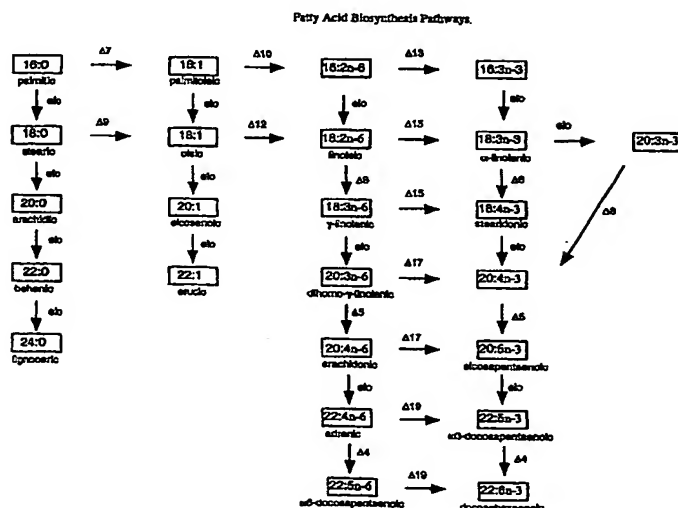




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(54) Title: ELONGASE GENES AND USES THEREOF



## (57) Abstract

The subject invention relates to the identification of four genes involved in the elongation of polyunsaturated acids (i.e., "elongases") and to uses thereof. Two of these genes are also involved in the elongation of monounsaturated fatty acids. In particular, elongase is utilized in the conversion of gamma linolenic acid (GLA) to dihogama linolenic acid (DGLA) and in the conversion of DGLA or 20:4n-3 to eicosapentaenoic acid (EPA). DGLA may be utilized in the production of polyunsaturated fatty acids, such as arachidonic acid (AA), docosahexaenoic acid (DHA), EPA, adrenic acid, ω6-docosapentaenoic acid or ω3-docosapentaenoic acid which may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.

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## ELONGASE GENES AND USES THEREOF

BACKGROUND OF THE INVENTIONTechnical Field

5       The subject invention relates to the identification of several genes involved in the elongation of long-chain polyunsaturated fatty acids (i.e., "elongases") and to uses thereof. In particular, the elongase enzyme is utilized in the conversion of one fatty acid to another. For example, elongase  
10       catalyzes the conversion of gamma linolenic acid (GLA) to dihomogamma-linolenic acid (DGLA, 20:3n-6) and the conversion of stearidonic acid (STA, 18:4n-3) to (n-3)-eicosatetraenoic acid (20:4n-3). Elongase also catalyzes the conversion of arachidonic acid (AA, 20:4n-6) to adrenic acid (ADA, 22:4n-6),  
15       the conversion of eicosapentaenoic acid (EPA, 20:5n-3) to  $\omega$ 3-docosapentaenoic acid (22:5n-3), and the conversion of  $\alpha$ -linolenic acid (ALA, 18:3n-3) to 20:3n-3. DGLA, for example, may be utilized in the production of other polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA) which may be added  
20       to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.

Background Information

25       The elongases which have been identified in the past differ in terms of the substrates upon which they act. Furthermore, they are present in both animals and plants. Those found in mammals have the ability to act on saturated, monounsaturated and polyunsaturated fatty acids. In contrast, those found in plants are specific for saturated or monounsaturated fatty  
30       acids. Thus, in order to generate polyunsaturated fatty acids in plants, there is a need for a PUFA-specific elongase.

In both plants and animals, the elongation process is believed to be the result of a four-step mechanism (Lassner et al., The Plant Cell 8:281-292 (1996)). CoA is the acyl carrier. Step one involves condensation of malonyl-CoA with a long-chain acyl-CoA to yield carbon dioxide and a  $\beta$ -ketoacyl-CoA in which the acyl moiety has been elongated by two carbon atoms. Subsequent reactions include reduction to  $\beta$ -hydroxyacyl-CoA, dehydration to an enoyl-CoA, and a second reduction to yield the elongated acyl-CoA. The initial condensation reaction is not only the substrate-specific step but also the rate-limiting step.

As noted previously, elongases, more specifically, those which utilize PUFAs as substrates, are critical in the production of long-chain polyunsaturated fatty acids which have many important functions. For example, PUFAs are important components of the plasma membrane of a cell where they are found in the form of phospholipids. They also serve as precursors to mammalian prostacyclins, eicosanoids, leukotrienes and prostaglandins. Additionally, PUFAs are necessary for the proper development of the developing infant brain as well as for tissue formation and repair. In view of the biological significance of PUFAs, attempts are being made to produce them, as well as intermediates leading to their production, efficiently.

A number of enzymes are involved in PUFA biosynthesis including elongases (elo) (see Figure 1). For example, linoleic acid (LA, 18:2- $\Delta$ 9,12 or 18:2n-6) is produced from oleic acid (OA, 18:1- $\Delta$ 9 or 18:1n-9) by a  $\Delta$ 12 desaturase. GLA (18:3- $\Delta$ 6,9,12) is produced from linoleic acid by a  $\Delta$ 6-desaturase. AA (20:4- $\Delta$ 5,8,11,14) is produced from dihomo- $\gamma$ -linolenic acid (DGLA,



20:3- $\Delta$ 8,11,14) by a  $\Delta$ 5-desaturase. As noted above, DGLA is produced from GLA by an elongase.

It must be noted that animals cannot desaturate beyond the  $\Delta$ 9 position and therefore cannot convert oleic acid into linoleic acid. Likewise,  $\alpha$ -linolenic acid (ALA, 18:3- $\Delta$ 9,12,15 or 18:3n-3) cannot be synthesized by mammals, since they lack  $\Delta$ 15 desaturase activity. However,  $\alpha$ -linolenic acid can be converted to stearidonic acid (STA, 18:4- $\Delta$ 6,9,12,15) by a  $\Delta$ 6-desaturase (see PCT publication WO 96/13591; see also U.S. Patent No. 5,552,306), followed by elongation to (n-3)-eicosatetraenoic acid (20:4- $\Delta$ 8,11,14,17 or 20:4n-3) in mammals and algae. This polyunsaturated fatty acid (i.e., 20:4- $\Delta$ 8,11,14,17) can then be converted to eicosapentaenoic acid (EPA, 20:5- $\Delta$ 5,8,11,14,17) by a  $\Delta$ 5-desaturase. Other eukaryotes, including fungi and plants, have enzymes which desaturate at carbons 12 (see PCT publication WO 94/11516 and U.S. Patent No. 5,443,974) and 15 (see PCT publication WO 93/11245). The major polyunsaturated fatty acids of animals therefore are either derived from diet and/or from desaturation and elongation of linoleic acid or  $\alpha$ -linolenic acid. In view of the inability of mammals to produce these essential long chain fatty acids, it is of significant interest to isolate genes involved in PUFA biosynthesis from species that naturally produce these fatty acids and to express these genes in a microbial, plant or animal system which can be altered to provide production of commercial quantities of one or more PUFAs. Consequently, there is a definite need for the elongase enzyme, the gene encoding the enzyme, as well as recombinant methods of producing this enzyme. Additionally, a need exists for oils containing levels of PUFA beyond those naturally present as well as those enriched in novel PUFAs. Such oils can only be made by isolation and expression of the elongase gene.

One of the most important long chain PUFAs, noted above, is arachidonic acid (AA). AA is found in filamentous fungi and can also be purified from mammalian tissues including the liver and the adrenal glands. As noted above, AA production from DGLA is catalyzed by a  $\Delta^5$ -desaturase, and DGLA production from  $\gamma$ -linolenic acid (GLA) is catalyzed by an elongase. However, until the present invention, no elongase had been identified which was active on substrate fatty acids in the pathways for the production of long chain PUFAs and, in particular, AA, eicosapentaenoic acid (EPA), adrenic acid, docosaheptaenoic acid (DHA, 22:6n-3),  $\omega$ 3-docosapentaenoic acid (22:5n-3) or  $\omega$ 6-docosapentaenoic acid (22:5n-6).

Two genes appeared to be of interest in the present search for the elongase gene. In particular, the jojoba  $\beta$ -ketoacyl-coenzyme A synthase (KCS), or jojoba KCS (GenBank Accession # U37088), catalyzes the initial reaction of the fatty acyl-CoA elongation pathway (i.e., the condensation of malonyl-CoA with long-chain acyl-CoA (Lassner et al., The Plant Cell 8:281-292 (1996))). Jojoba KCS substrate preference is 18:0, 20:0, 20:1, 18:1, 22:1, 22:0 and 16:0. Saccharomyces cerevisiae elongase (ELO2) also catalyzes the conversion of long chain saturated and monounsaturated fatty acids, producing high levels of 22:0, 24:0, and also 18:0, 18:1, 20:0, 20:1, 22:0, 22:1, and 24:1 (Oh et al., The Journal of Biological Chemistry 272 (28):17376-17384 (1997); see also U.S. Patent No. 5,484,724 for a nucleotide sequence which includes the sequence of ELO2; see PCT publication WO 88/07577 for a discussion of the sequence of a glycosylation inhibiting factor which is described in Example V). The search for a long chain PUFA-specific elongase in Mortierella alpina began based upon a review of the homologies shared between these two genes and by expression screening for PUFA-elongase activity.

SUMMARY OF THE INVENTION

The present invention relates to an isolated nucleotide  
5 sequence corresponding to or complementary to at least about 50%  
of the nucleotide sequence shown in SEQ ID NO:1 (Figure 6).  
This isolated sequence may be represented by SEQ ID NO:1. The  
sequence encodes a functionally active elongase which utilizes a  
polyunsaturated fatty acid or a monounsaturated fatty acid as a  
10 substrate. In particular, the sequence may be derived from a  
fungus of the genus Mortierella and may specifically be isolated  
from Mortierella alpina.

The present invention also includes a purified protein  
encoded by the above nucleotide sequence as well as a purified  
15 polypeptide which elongates polyunsaturated fatty acids or  
monounsaturated fatty acids and has at least about 50% amino  
acid similarity to the amino acid sequence of the purified  
protein encoded by the above nucleotide sequence.

Additionally, the present invention encompasses a method of  
20 producing an elongase enzyme comprising the steps of: a)  
isolating the nucleotide sequence represented by SEQ ID NO:1  
(Figure 6); b) constructing a vector comprising: i) the isolated  
nucleotide sequence operably linked to ii) a promoter; and c)  
introducing the vector into a host cell under time and  
25 conditions sufficient for expression of the elongase enzyme.  
The host cell may be a eukaryotic cell or a prokaryotic cell.

The prokaryotic cell may be, for example an E. coli cell, a  
cyanobacterial cell, or a B. subtilis cell. The eukaryotic cell  
may be, for example, a mammalian cell, an insect cell, a plant  
30 cell or a fungal cell. The fungal cell may be, for example,  
Saccharomyces spp., Candida spp., Lipomyces spp., Yarrowia spp.,  
Kluyveromyces spp., Hansenula spp., Aspergillus spp.,

Penicillium spp., Neurospora spp., Trichoderma spp. or Pichia spp. In particular, the fungal cell may be a yeast cell such as Saccharomyces spp., in particular, Saccharomyces cerevisiae, Candida spp., Hansenula spp. or Pichia spp.

5 The invention also includes a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:1 (Figure 6) operably linked to b) a promoter, as well as a host cell comprising this vector. The host may be a prokaryotic cell or a eukaryotic cell. Suitable examples of prokaryotic cells include  
10 E. coli, Cyanobacteria, and B. subtilis cells. Suitable examples of eukaryotic cells include a mammalian cell, an insect cell, a plant cell and a fungal cell. The fungal cell may be, for example, Saccharomyces spp., Candida spp., Lipomyces spp., Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus  
15 spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp. In particular, the fungal cell may be, for example, a yeast cell such as, for example, Saccharomyces spp., in particular, Saccharomyces cerevisiae, Candida spp., Hansenula spp. and Pichia spp.

20 The present invention includes a plant cell, plant or plant tissue comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of at least one fatty acid selected from the group consisting of a monounsaturated fatty acid and a polyunsaturated fatty acid by  
25 the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, dihomo- $\gamma$ -linolenic acid (DGLA), 20:4n-3, and adrenic acid (ADA). The invention also includes one or more plant oils or fatty acids expressed by the plant cell, plant or plant tissue. Additionally, the present  
30 invention encompasses a transgenic plant comprising the above-described vector, wherein expression of the nucleotide sequence

of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

Furthermore, the present invention includes a transgenic, non-human mammal whose genome comprises a DNA sequence encoding an elongase operably linked to a promoter. The DNA sequence may be represented by SEQ ID NO:1 (Figure 6). The present invention also includes a fluid (e.g., milk) produced by the transgenic, non-human wherein the fluid comprises a detectable level of at least one elongase or products thereof such as, for example, DGLA,  $\omega$ 6-docosapentaenoic acid, ADA and/or 20:4n-3 (see Figure 1).

Additionally, the present invention includes a method for producing a polyunsaturated fatty acid comprising the steps of: a) isolating said nucleotide sequence represented by SEQ ID NO:1 (Figure 6); b) constructing a vector comprising the isolated nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of elongase enzyme encoded by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a "substrate" polyunsaturated fatty acid in order to convert the substrate to a "product" polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be selected from the group consisting of, for example,  $\gamma$ -linolenic acid (GLA), stearidonic acid (STA) and arachidonic acid (AA), and the product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3, and ADA, respectively. The method may further comprise the step of exposing the product polyunsaturated fatty acid to at least one desaturase in order to convert the product polyunsaturated fatty acid to "another" polyunsaturated fatty acid. The product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3, and ADA. The another

polyunsaturated fatty acid may be selected from the group consisting of, for example, AA, eicosapentaenoic acid (EPA),  $\omega$ 6-docosapentaenoic acid, respectively, and the at least one desaturase is  $\Delta$ 5-desaturase, with respect to production of AA or EPA, and  $\Delta$ 4-desaturase, with respect to production of  $\omega$ 6-docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a "final" polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, docosahexaenoic acid (DHA), AA,  $\omega$ 6-docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid.

Also, the present invention includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method. The product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3 and ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid. The nutritional composition may be, for example, an infant formula, a dietary supplement or a dietary substitute and may be administered to a human or an animal and may be administered enterally or parenterally. The nutritional composition may further comprise at least one macronutrient selected from the

group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, triglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, protein hydrolysates, sunflower oil, safflower oil, corn oil, and flax oil. It may also comprise at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.

Additionally, the present invention encompasses a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method of claim 32, and the final polyunsaturated fatty acid produced according to the above-described method and 2) a pharmaceutically acceptable carrier. The composition may be administered to a human or an animal. It may also further comprise at least one element selected from the group consisting of a vitamin, a mineral, a salt, a carbohydrate, an amino acid, a free fatty acid, a preservative, an excipient, an anti-histamine, a growth factor, an antibiotic, a diluent, a phospholipid, an antioxidant, and a phenolic compound. It may be administered enterally, parenterally, topically, rectally, intramuscularly, subcutaneously, intradermally, or by any other appropriate means.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another

polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, and ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid.

Moreover, the present invention also includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method.

Additionally, the present invention includes a method of preventing or treating a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient the above nutritional composition in an amount sufficient to effect prevention or treatment.

The present invention also includes an isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO:2 (Figure 22). This sequence may be represented by SEQ ID NO:2. The sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate. This sequence may also be derived, for example, from a fungus of the genus Mortierella. In particular, it may be derived from M. alpina.

Additionally, the present invention includes a purified protein encoded by the above nucleotide sequence as well as a



purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of the purified protein.

5 The present invention also includes a method of producing an elongase enzyme as described above. The sequence inserted in the vector is represented by SEQ ID NO:2 (Figure 22). The host cell may be prokaryotic or eukaryotic. Suitable examples are described above.

10 The present invention also includes a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:2 (Figure 22) operably linked to b) a promoter, as well as a host cell comprising this vector. Again, the host cell may be eukaryotic or prokaryotic. Suitable examples are described above.

15 The invention also includes a plant cell, plant or plant tissue comprising the above vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid by the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. Additionally, the invention includes one  
20 or more plant oils or fatty acids expressed by the plant cell, plant or plant tissue.

Furthermore, the present invention also includes a transgenic plant comprising the above vector, wherein expression of the nucleotide sequence (SEQ ID NO:2) of the vector results  
25 in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

The invention also includes a transgenic, non-human mammal whose genome comprises a DNA sequence (SEQ ID NO:2) encoding an elongase operably linked to a promoter. The invention also  
30 includes a fluid produced by this transgenic, non-human mammal wherein the fluid comprises a detectable level of at least one elongase or products thereof.

The present invention also includes a method for producing a polyunsaturated fatty acid comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:2 (Figure 22); b) constructing a vector comprising the isolated nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of an elongase enzyme encoded by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, GLA, STA, or AA, the product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or  $\omega$ 6-docosapentaenoic acid, respectively. The method may further comprise the step of exposing the expressed elongase enzyme to at least one desaturase in order to convert the product polyunsaturated fatty acid to another polyunsaturated fatty acid. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA, the another polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-docosapentaenoic acid, respectively, and the at least one desaturase is  $\Delta$ 5-desaturase with respect to production of AA or EPA, and  $\Delta$ 4-desaturase with respect to production of  $\omega$ 6-docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, docosahexaenoic acid, AA,  $\omega$ 6-docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid.

The invention also includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the product polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2, the another  
5 polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2. The product polyunsaturated fatty acid may be selected from the group  
10 consisting of, for example, DGLA, 20:4n-3 and ADA. The another polyunsaturated fatty acid may be selected from the group consisting of, for example, AA, EPA, and  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be selected from the group consisting of, for example, DHA, AA,  $\omega$ 6-  
15 docosapentaenoic acid, and  $\omega$ 3-docosapentaenoic acid. The other attributes of the composition are the same as those described above with respect to administration, characterization, components, etc.

The present invention also includes a pharmaceutical  
20 composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method of noted above with respect to SEQ ID NO:2, the another polyunsaturated fatty acid produced according to the method  
25 described above with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and 2) a pharmaceutically acceptable carrier. The characteristics of the above-described pharmaceutical composition (e.g.,  
30 administration, components, etc.) also apply to this composition.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2, the another polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 or ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid.

The invention also includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, the another polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2.

Additionally, the present invention includes a method of preventing or treating a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient the nutritional composition described directly above in an amount sufficient to effect the prevention or treatment.

Furthermore, the present invention includes an isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO:3 (Figure 43). This sequence may be that represented by SEQ ID

NO:3. This sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid or a monounsaturated fatty acid as a substrate. The sequence is derived from a mammal such as, for example, a human.

5 The invention also includes a purified protein encoded by this nucleotide sequence. Also, the invention includes a purified polypeptide which elongates polyunsaturated fatty acids or monounsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of this purified  
10 protein.

Additionally, the invention includes method of producing an elongase enzyme comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:3 (Figure 43); b) constructing a vector comprising: i) the isolated nucleotide  
15 sequence operably linked to ii) a promoter; and c) introducing said vector into a host cell under time and conditions sufficient for expression of the elongase enzyme. The host cell may be the same as that described above with respect to the corresponding methods utilizing SEQ ID NO:1 or 2.

20 The invention also includes a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:3 (Figure 43) operably linked to b) a promoter, as well as a host cell comprising this vector. The host cell may be the same as that described above.

The invention also includes a plant cell, plant or plant  
25 tissue comprising the above-described vector comprising SEQ ID NO:3, wherein expression of the nucleotide sequence of the vector results in production of at least one fatty acid selected from the group consisting of a monounsaturated fatty acid and a polyunsaturated fatty acid by said plant cell, plant or plant  
30 tissue. The polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 or ADA. The invention also includes one or more

plant oils or acids expressed by the plant cell, plant or plant tissue.

The invention also includes a transgenic plant comprising the vector comprising SEQ ID NO:3, wherein expression of the  
5 nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

Additionally, the present invention includes a transgenic, non-human mammal whose genome comprises a human DNA sequence encoding an elongase operably linked to a promoter. The DNA  
10 sequence is represented by SEQ ID NO:3 (Figure 43). The invention also includes a fluid produced by said transgenic, non-human mammal wherein said fluid comprises a detectable level of at least one elongase or products thereof.

The invention also encompasses a method for producing a  
15 polyunsaturated fatty acid comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:3 (Figure 43); b) constructing a vector comprising said nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of elongase enzyme encoded  
20 by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, GLA, STA or AA, and the product  
25 polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA, respectively. The method may further comprise the step of exposing the product polyunsaturated fatty acid to at least one desaturase in order to convert the product polyunsaturated fatty acid to another polyunsaturated fatty acid. The product  
30 polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 and ADA, the another polyunsaturated fatty acid may be, for example, AA, EPA, and  $\omega$ 6-docosapentaenoic acid, respectively,

and the at least one desaturase is  $\Delta 5$ -desaturase with respect to production of AA or EPA and  $\Delta 4$ -desaturase with respect to production of  $\omega 6$ -docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA,  $\omega 6$ -docosapentaenoic acid, and  $\omega 3$ -docosapentaenoic acid.

The nutritional composition comprising at least one polyunsaturated fatty acid which may be, for example, product polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3, another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The another polyunsaturated fatty acid may be selected from the group consisting of AA, EPA, or  $\omega 6$ -docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA,  $\omega 6$ -docosapentaenoic acid, or  $\omega 3$ -docosapentaenoic acid. The other properties or characteristic of the nutritional composition (e.g., administration, components, etc.) as the same as those recited above with respect to the other nutritional compositions.

Moreover, the present invention also includes a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to

the method described above in connection with SEQ ID NO:3, the another polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:3 and 2) a pharmaceutically acceptable carrier. The other properties of the composition (e.g., administration, additional components, etc.) are the same as those recited above with respect to the other pharmaceutical compositions.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3, the another polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid.

Also, the present invention includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3, said another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3.

A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising



administering to the patient the nutritional composition recited above in connection with SEQ ID NO:3 in an amount sufficient to effect the prevention or treatment.

Additionally, the present invention includes an isolated  
5 nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO:4 (Figure 46). The sequence may be represented by SEQ ID NO:4. It encodes a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate. The sequence may be  
10 derived or isolated from a nematode of the genus Caenorhabditis and, in particular, may be isolated from C. elegans.

The present invention includes a purified protein encoded by the nucleotide sequence above. The invention also includes a purified polypeptide which elongates polyunsaturated fatty acids  
15 and has at least about 30% amino acid similarity to the amino acid sequence of the purified protein.

Additionally, the present invention includes a method of producing an elongase enzyme comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:4  
20 (Figure 46); b) constructing a vector comprising: i) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing the vector into a host cell under time and conditions sufficient for expression of the elongase enzyme. The properties of the host cell are the same as those described  
25 above in connection with SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

The present include also encompasses a vector comprising:  
a) a nucleotide sequence as represented by SEQ ID NO:4 (Figure 46) operably linked to b) a promoter, as well as a host cell  
30 comprising this vector. The host cell has the same properties as those recited above in connection with the host cell recited above for SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

Moreover, the present invention includes a plant cell, plant or plant tissue comprising the above vector comprising SEQ ID NO:4, wherein expression of said nucleotide sequence of the vector results in production of a polyunsaturated fatty acid by the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The invention also includes one or more plant oils or fatty acids expressed by this plant cell, plant or plant tissue.

The invention also includes transgenic plant comprising the above vector including the nucleotide sequence corresponding to SEQ ID NO:4, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

Additionally, the present invention includes a transgenic, non-human mammal whose genome comprises a C. elegans DNA sequence encoding an elongase operably linked to a promoter. The DNA sequence may be represented by SEQ ID NO:4 (Figure 46). The invention also includes a fluid produced by the transgenic, non-human mammal of claim 187 wherein the fluid comprises a detectable level of at least one elongase or products thereof.

The invention also includes a method for producing a polyunsaturated fatty acid comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:4 (Figure 46); b) constructing a vector comprising the isolated nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of an elongase enzyme encoded by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, GLA, STA, or AA, and the product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3,

or ADA, respectively. The method may further comprise the step of exposing the expressed elongase enzyme to at least one desaturase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 or ADA, the another polyunsaturated fatty acid may be, for example, AA, EPA or  $\omega$ 6-docosapentaenoic acid, respectively, and the at least one desaturase is  $\Delta$ 5-desaturase with respect to production of AA or EPA, and  $\Delta$ 4-desaturase with respect to production of  $\omega$ 6-docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA,  $\omega$ 6-docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid.

The invention also includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said the polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4, and the final polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA,  $\omega$ 6-docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid. The other characteristics of the

composition are the same as those recited for the nutritional compositions present above.

Additionally, the present invention includes a pharmaceutical composition comprising 1) at least one  
5 polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4, and the  
10 final polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4 and 2) a pharmaceutically acceptable carrier. The composition has the same properties (e.g., administration, added elements, etc.) as those described above with respect to the other pharmaceutical  
15 compositions.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method described above in connection  
20 with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4, and the final polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4. The product polyunsaturated fatty acid may  
25 be, for example, DGLA, 20:4n-3 or ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA or  $\omega$ 6-docosapentaenoic acid. The polyunsaturated fatty acid may be, for example, DHA, ADA,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid.

30 Additionally, the present invention includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced

according to the method recited above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4 and the final polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4.

Furthermore, the present invention encompasses a method of preventing or treating a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient the nutritional composition recited with respect to SEQ ID NO:4 in an amount sufficient to effect the treatment or prevention.

All U.S. patents and publications referred to herein are hereby incorporated in their entirety by reference.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents various fatty acid biosynthesis pathways. The role of the elongase enzyme (elo) should be noted.

Figure 2 represents the percent similarity and percent identity between the amino acid sequences of jojoba KCS and ELO2.

Figure 3 represents the S. cerevisiae ELO2 sequence homologous to the jojoba KCS sequence (primer sequence underlined) of Figure 2.

Figure 4A shows the physical map of pRAE-2 containing the MAELO cDNA. Figure 4B represents the physical map of the constitutive expression vector, pRAE-5, used for elongase enzyme production in yeast.

Figure 5 represents a comparison of the nucleotide sequences of clones pRAE-5 and pRAE-6.

Figure 6 illustrates the complete nucleotide sequence of Mortierella alpina elongase (MAELO).

Figure 7 represents the amino acid sequence of the Mortierella alpina elongase translated from MAELO (see Figure 6).

Figure 8 represents an amino acid sequence alignment among 3 elongases: S. cerevisiae ELO2 (GNS1), S. cerevisiae ELO3 (SUR4) and the translated MAELO sequence as shown in Figure 7.

Figure 9 represents a comparison between the nucleotide sequence MAELO and the nucleotide sequence of ELO2 from S. cerevisiae.

Figures 10A and 10B represents the PUFA elongase activity of MAELO expressed in baker's yeast.

Figure 11 illustrates the PUFA elongase activity of MAELO when co-expressed with the  $\Delta 5$ -desaturase cDNA from M. alpina to produce AA.

Figure 12 compares the PUFA elongase activity of MAELO to the overexpression of ELO2 from S. cerevisiae in baker's yeast.

Figures 13, 14 and 15 represent three separate comparisons of amino acid sequences derived from C. elegans nucleotide sequences in the GenEMBL database with the translated MAELO.

Figure 16 shows the comparison between amino acid translations of two different mammalian sequences in the GenEMBL database and the translated MAELO.

Figure 17 shows the comparison of a translated DNA sequence (see published PCT application WO 88/07577) with the amino acid sequence derived from MAELO, which was detected during a database search.

Figure 18 shows the complete nucleotide sequence of the  $\Delta 5$ -desaturase from M. alpina.

Figure 19 represents the initial GC-FAME analysis of MAD708 pool. The detection of a DGLA (C20:3n-6) peak should be noted.

Figure 20 represents the PUFA elongase activity of the five MAD708 clones in yeast with GLA as substrate. All clones have apparent elongase activity.

5 Figure 21 represents the DNA sequencing analysis of plasmid pRPB2. The analysis reveals an open reading frame of 957 bp in length.

Figure 22 shows the complete nucleotide sequence of the M. alpina cDNA, contained in the plasmid pRPB2, which is designated GLELO for its GLA elongase activity.

10 Figure 23 represents the amino acid sequence of the M. alpina elongase translated from GLELO (see Figure 22).

Figure 24 illustrates the n-6 PUFA elongase activity in an induced culture of 334(pRPB2) when supplemented with GLA.

15 Figure 25 represents the n-3 and n-6 PUFA elongase activity in an induced culture of 334(pRPB2) when supplemented with 25  $\mu$ m of other fatty acid substrates.

Figure 26A illustrates the elongase activity of GLELO with GLA as a substrate when co-expressed with the M. alpina  $\Delta$ 5-desaturase cDNA to produce AA. Figure 26B illustrates the  
20 elongase activity of GLELO with STA as a substrate when co-expressed with the M. alpina  $\Delta$ 5-desaturase cDNA to produce EPA.

Figure 27 illustrates the comparison between the translated GLELO sequence (see Figure 23) and the translated MAELO sequence (see Figure 7).

25 Figure 28 represents a comparison of the amino acid sequence of 4 elongases: the translated amino acid sequence of GLELO (see Figure 23), MAELO (see Figure 7), S. cerevisiae ELO2 (GNS1), and S. cerevisiae ELO3 (SUR4). The histidine box is underlined.

30 Figure 29 represents an alignment between translated MAELO sequence and translated putative human homologue HS1 sequence.

Figure 30 represents an alignment between the translated MAELO sequence and the translated putative human homologue HS2 sequence.

Figure 31 shows an alignment between the translated MAELO  
5 sequence and the translated putative mouse homologue MM2 sequence.

Figure 32 represents an alignment between the translated MAELO and the translated putative mouse homologue AI225632 sequence.

10 Figure 33 illustrates an alignment between the translated GLELO sequence and the translated human homologue AI815960 sequence.

Figure 34 shows an alignment between the translated GLELO  
15 sequence and the translated putative human homologue HS1 sequence.

Figure 35 represents an alignment between the translated GLELO sequence and the translated putative human homologue sequence from AC004050.

Figure 36 illustrates an alignment between the translated  
20 GLELO sequence and the translated putative mouse homologue MM2 sequence.

Figure 37 represents an alignment of the translated GLELO sequence and a translated putative mouse homologue AI225632 sequence.

25 Figure 38 illustrates an alignment of the translated GLELO sequence and a translated putative mouse homologue U97107.

Figure 39 represents an alignment of the translated GLELO sequence and a translated putative C. elegans U68749 (F56H11.4) homologue sequence.

30 Figure 40 shows an alignment between the translated MAELO sequence and a translated putative C. elegans U68749 (F56H11.4) homologue sequence.



Figure 41 represents an alignment between the translated GLELO sequence and a translated putative Drosophila melanogaster homologue sequence, DM1.

Figure 42 illustrates an alignment between the translated MAELO sequence and a translated putative Drosophila melanogaster homologue sequence, DM1.

Figure 43 illustrates the complete nucleotide sequence of a human elongase HSELO1.

Figure 44 represents the deduced amino acid sequence of the human elongase HSELO1.

Figure 45 illustrates the elongase activity (PUFA and others) of an induced culture of 334(pRAE-58-A1) when supplemented with GLA or AA.

Figure 46 shows the complete nucleotide sequence of the C. elegans elongase CELO.

Figure 47 represents the deduced amino acid of C. elegans elongase CELO.

Figure 48 illustrates the PUFA elongase activity of an induced culture of 334(pRET-21) and 334(pRET-22) when supplemented with GLA and AA.

Figure 49 represents the complete nucleotide sequence of the putative human elongase gene HS3.

Figure 50 illustrates the deduced amino acid sequence of the putative human elongase enzyme HS3.

#### DETAILED DESCRIPTION OF THE INVENTION

The subject invention relates to nucleotide and corresponding amino acid sequences of two elongase cDNAs derived from Mortierella alpina, as well as to nucleotide and corresponding amino acid sequences of an elongase cDNA derived from a human and one derived from C. elegans. Furthermore, the

subject invention also includes uses of the cDNAs and of the proteins encoded by the genes. For example, the genes and corresponding enzymes may be used in the production of polyunsaturated fatty acids and/or monounsaturated fatty acids such as DGLA, AA, ADA, EPA and/or DHA which may be added to pharmaceutical compositions, nutritional compositions and to other valuable products.

#### The Elongase Genes and Enzymes Encoded Thereby

As noted above, an elongase enzyme encoded by an elongase cDNA is essential in the production of various polyunsaturated fatty acids, in particular, 20-24 carbon PUFAs. With respect to the present invention, the nucleotide sequence of the isolated M. alpina elongase cDNA (MAELO) is shown in Figure 6, and the amino acid sequence of the corresponding purified protein or enzyme encoded by this nucleotide sequence is shown in Figure 7. Additionally, the nucleotide sequence of the isolated GLA elongase cDNA (GLELO) is shown in Figure 22, and the amino acid sequence of the corresponding purified protein or enzyme encoded by this nucleotide sequence is shown in Figure 23. The nucleotide sequence of the isolated human sequence 1 (HSELO1) elongase is shown in Figure 43, and the amino acid sequence of the corresponding purified protein or enzyme encoded by this sequence is shown in Figure 44. Furthermore, the nucleotide sequence of the isolated C. elegans elongase cDNA (CEELO1) is shown in Figure 46, and the amino acid sequence of the corresponding purified protein or enzyme encoded thereby is shown in Figure 47.

As an example, the isolated elongases encoded by the cDNAs of the present invention elongate GLA to DGLA or elongate STA to 20:4n-3 or elongate AA to ADA. The production of arachidonic acid from DGLA, or EPA from 20:4n-3, is then catalyzed by a  $\Delta 5$ -

desaturase. Thus, neither AA (or EPA), nor DGLA (or 20:4n-3) nor ADA (or  $\omega$ 3-docosapentaenoic acid), can be synthesized without at least one elongase cDNA and enzyme encoded thereby.

It should be noted that the present invention also  
5 encompasses nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 50%, preferably at least about 60%, and more preferably at least about 70% of the nucleotides in SEQ ID NO:1 (i.e., the nucleotide sequence of  
10 the MAELO cDNA described herein (see Figure 6)). Furthermore, the present invention also includes nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 35%, preferably at least about 45%, and more  
15 preferably at least about 55% of the nucleotides in SEQ ID NO:2 (i.e., the nucleotide sequence of the GLELO cDNA described herein (see Figure 22)). Additionally, the present invention also includes nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e.,  
20 having identity to) or complementary to at least about 35%, preferably at least about 45%, and more preferably at least about 55% of the nucleotides in SEQ ID NO:3 (i.e., the nucleotide sequence of the human sequence 1 (HSELO1) cDNA described herein (see Figure 43)). In addition, the present  
25 invention also includes nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 35%, preferably at least about 45%, and more preferably at least about 55% of the nucleotides in SEQ ID NO:4 (i.e., the  
30 nucleotide sequence of the C. elegans cDNA, CELO1, described herein (see Figure 46)). Such sequences may be derived from non-Mortierella sources (e.g., a eukaryote (e.g., Thraustochytrium

spp. (e.g., Thraustochytrium aureum and Thraustochytrium roseum), Schizochytrium spp. (e.g., Schizochytrium aggregatum), Conidiobolus spp. (e.g., Conidiobolus nanodes), Entomorphthora spp. (e.g., Entomorphthora exitalis), Saprolegnia spp. (e.g., Saprolegnia parasitica and Saprolegnia diclina), Leptomit spp. (e.g., Leptomit lacteus), Entomophthora spp., Pythium spp., Porphyridium spp. (e.g., Porphyridium cruentum), Conidiobolus spp., Phytophthora spp., Penicillium spp., Coidosporium spp., Mucor spp. (e.g., Mucor circinelloides and Mucor javanicus), Fusarium spp., Aspergillus spp. and Rhodotorula spp.), a yeast (e.g., Dipodascopsis uninucleata), a non-mammalian organism such as a fly (e.g., Drosophila melanogaster) or Caenorhabditis spp. (e.g., Caenorhabditis elegans), or a mammal (e.g., a human or a mouse). Such sequences may be derived from species within the genus Mortierella, other than the species alpina, for example, Mortierella elongata, Mortierella exigua, Mortierella isabellina, Mortierella hygrophila, and Mortierella ramanniana, va. angulispora. Furthermore, the present invention also encompasses fragments and derivatives of the nucleotide sequences of the present invention (i.e., SEQ ID NO:1 (MAELO), SEQ ID NO:2 (GLELO), SEQ ID NO:3 (HSELO1) and SEQ ID NO:4 (CEELO1)), as well as of the sequences derived from non-Mortierella sources and having the above-described complementarity or correspondence/identity. Functional equivalents of the above-sequences (i.e., sequences having elongase activity) are also encompassed by the present invention.

For purposes of the present invention, "complementarity" is defined as the degree of relatedness between two DNA segments. It is determined by measuring the ability of the sense strand of one DNA segment to hybridize with the antisense strand of the other DNA segment, under appropriate conditions, to form a

double helix. In the double helix, wherever adenine appears in one strand, thymine appears in the other strand. Similarly, wherever guanine is found in one strand, cytosine is found in the other. The greater the relatedness between the nucleotide sequences of two DNA segments, the greater the ability to form hybrid duplexes between the strands of two DNA segments.

"Identity" between two nucleotide sequences is defined as the degree of sameness, correspondence or equivalence between the same strands (either sense or antisense) of two DNA segments. The greater the percent identity, the higher the correspondence, sameness or equivalence between the strands.

"Similarity" between two amino acid sequences is defined as the presence of a series of identical as well as conserved amino acid residues in both sequences. The higher the degree of similarity between two amino acid sequences, the higher the correspondence, sameness or equivalence of the two sequences. ("Identity" between two amino acid sequences is defined as the presence of a series of exactly alike or invariant amino acid residues in both sequences.)

The definitions of "complementarity", "identity", and "similarity" are well known to those of ordinary skill in the art.

The invention also includes a purified polypeptide which elongates polyunsaturated and monounsaturated fatty acids and has at least about 50% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g., Figure 7 (MAELO)) and which are, in turn, encoded by the above-described nucleotide sequences. Additionally, the present invention includes a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g., Figure 23 (GLELO)) and which are, in turn, encoded by the above-

described nucleotide sequences. Furthermore, the invention also includes a purified polypeptide which elongates polyunsaturated and monounsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g., Figure 44 (HSELO1)) and which are, in turn, encoded by the above-described nucleotide sequences. Also, the present invention includes a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g., Figure 47 (CEELO1)) and which are, in turn, encoded by the above-described nucleotide sequences.

The present invention also encompasses an isolated nucleotide sequence which encodes PUFA elongase activity and that is hybridizable, under moderately stringent conditions, to a nucleic acid having a nucleotide sequence corresponding or complementary to the nucleotide sequence represented by SEQ ID NO:1 shown in Figure 6 (MAELO) and/or SEQ ID NO:2 shown in Figure 22 (GLELO) and/or SEQ ID NO:3 (HSELO1) shown in Figure 43 and/or SEQ ID NO:4 (CEELO1) shown in Figure 46. A nucleic acid molecule is "hybridizable" to another nucleic acid molecule when a single-stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and ionic strength (see Sambrook et al., "Molecular Cloning: A Laboratory Manual, Second Edition (1989), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York)). The conditions of temperature and ionic strength determine the "stringency" of the hybridization.

"Hybridization" requires that two nucleic acids contain complementary sequences. However, depending on the stringency of the hybridization, mismatches between bases may occur. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of

complementarity. Such variables are well known in the art. More specifically, the greater the degree of similarity or homology between two nucleotide sequences, the greater the value of  $T_m$ , melting temperature, for hybrids of nucleic acids having those sequences. For hybrids of greater than 100 nucleotides in length, equations for calculating  $T_m$  have been derived (see Sambrook et al., supra). For hybridization with shorter nucleic acids, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., supra).

#### Production of the Elongase Enzyme

Once the gene encoding the elongase has been isolated, it may then be introduced into either a prokaryotic or eukaryotic host cell through the use of a vector, plasmid or construct.

The vector, for example, a bacteriophage, cosmid or plasmid, may comprise the nucleotide sequence encoding the elongase as well as any promoter which is functional in the host cell and is able to elicit expression of the elongase encoded by the nucleotide sequence. The promoter is in operable association with or operably linked to the nucleotide sequence. (A promoter is said to be "operably linked" with a coding sequence if the promoter affects transcription or expression of the coding sequence.) Suitable promoters include, for example, those from genes encoding alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglucosomerase, phosphoglycerate kinase, acid phosphatase, T7, TP1, lactase, metallothionein, cytomegalovirus immediate early, whey acidic protein, glucoamylase, and promoters activated in the presence of galactose, for example, GAL1 and GAL10. Additionally, nucleotide sequences which encode other proteins, oligosaccharides, lipids, etc. may also be included within the

vector as well as other regulatory sequences such as a polyadenylation signal (e.g., the poly-A signal of SV-40T-antigen, ovalalbumin or bovine growth hormone). The choice of sequences present in the construct is dependent upon the desired expression products as well as the nature of the host cell.

As noted above, once the vector has been constructed, it may then be introduced into the host cell of choice by methods known to those of ordinary skill in the art including, for example, transfection, transformation and electroporation (see Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., Vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press (1989)). The host cell is then cultured under suitable conditions permitting expression of the PUFA which is then recovered and purified.

It should also be noted that one may design a unique triglyceride or oil if one utilizes one construct or vector comprising the nucleotide sequences of two or more cDNAs (e.g., MAELO, GLELO, HSELO1 and/or CELO1). This vector may then be introduced into one host cell. Alternatively, each of the sequences may be introduced into a separate vector. These vectors may then be introduced into two host cells, respectively, or into one host cell.

Examples of suitable prokaryotic host cells include, for example, bacteria such as Escherichia coli, Bacillus subtilis as well as cyanobacteria such as Spirulina spp. (i.e., blue-green algae). Examples of suitable eukaryotic host cells include, for example, mammalian cells, plant cells, yeast cells such as Saccharomyces spp., Lipomyces spp., Candida spp. such as Yarrowia (Candida) spp., Kluyveromyces spp., Pichia spp., Trichoderma spp. or Hansenula spp., or fungal cells such as filamentous fungal cells, for example, Aspergillus, Neurospora



and Penicillium. Preferably, Saccharomyces cerevisiae (baker's yeast) cells are utilized.

Expression in a host cell can be accomplished in a transient or stable fashion. Transient expression can occur  
5 from introduced constructs which contain expression signals functional in the host cell, but which constructs do not replicate and rarely integrate in the host cell, or where the host cell is not proliferating. Transient expression also can be accomplished by inducing the activity of a regulatable  
10 promoter operably linked to the gene of interest, although such inducible systems frequently exhibit a low basal level of expression. Stable expression can be achieved by introduction of a construct that can integrate into the host genome or that autonomously replicates in the host cell. Stable expression of  
15 the gene of interest can be selected for through the use of a selectable marker located on or transfected with the expression construct, followed by selection for cells expressing the marker. When stable expression results from integration, the site of the construct's integration can occur randomly within  
20 the host genome or can be targeted through the use of constructs containing regions of homology with the host genome sufficient to target recombination with the host locus. Where constructs are targeted to an endogenous locus, all or some of the transcriptional and translational regulatory regions can be  
25 provided by the endogenous locus.

A transgenic mammal may also be used in order to express the enzyme of interest (i.e., the elongase) encoded by one or both of the above-described nucleotide sequences. More specifically, once the above-described construct is created, it  
30 may be inserted into the pronucleus of an embryo. The embryo may then be implanted into a recipient female. Alternatively, a nuclear transfer method could also be utilized (Schnieke et al.,

Science 278:2130-2133 (1997)). Gestation and birth are then permitted to occur(see, e.g., U.S. Patent No. 5,750,176 and U.S. Patent No. 5,700,671). Milk, tissue or other fluid samples from the offspring should then contain altered levels of PUFAs, as compared to the levels normally found in the non-transgenic animal. Subsequent generations may be monitored for production of the altered or enhanced levels of PUFAs and thus incorporation of the gene or genes encoding the elongase enzyme into their genomes. The mammal utilized as the host may be selected from the group consisting of, for example, a mouse, a rat, a rabbit, a pig, a goat, a sheep, a horse and a cow. However, any mammal may be used provided it has the ability to incorporate DNA encoding the enzyme of interest into its genome.

For expression of an elongase polypeptide, functional transcriptional and translational initiation and termination regions are operably linked to the DNA encoding the elongase polypeptide. Transcriptional and translational initiation and termination regions are derived from a variety of nonexclusive sources, including the DNA to be expressed, genes known or suspected to be capable of expression in the desired system, expression vectors, chemical synthesis, or from an endogenous locus in a host cell. Expression in a plant tissue and/or plant part presents certain efficiencies, particularly where the tissue or part is one which is harvested early, such as seed, leaves, fruits, flowers, roots, etc. Expression can be targeted to that location with the plant by utilizing specific regulatory sequence such as those of U.S. Patent Nos. 5,463,174, 4,943,674, 5,106,739, 5,175,095, 5,420,034, 5,188,958, and 5,589,379. Alternatively, the expressed protein can be an enzyme which produces a product which may be incorporated, either directly or upon further modifications, into a fluid fraction from the host plant. Expression of an elongase gene or genes, or antisense

elongase transcripts, can alter the levels of specific PUFAs, or derivatives thereof, found in plant parts and/or plant tissues. The elongase polypeptide coding region may be expressed either by itself or with other genes, in order to produce tissues and/or plant parts containing higher proportions of desired PUFAs or in which the PUFA composition more closely resembles that of human breast milk (Prieto et al., PCT publication WO 95/24494). The termination region may be derived from the 3' region of the gene from which the initiation region was obtained or from a different gene. A large number of termination regions are known to and have been found to be satisfactory in a variety of hosts from the same and different genera and species. The termination region usually is selected as a matter of convenience rather than because of any particular property.

As noted above, a plant (e.g., Glycine max (soybean) or Brassica napus (canola)), plant tissue, corn, potatoe, sunflower, safflower or flax may also be utilized as a host or host cell, respectively, for expression of the elongase enzyme(s) which may, in turn, be utilized in the production of polyunsaturated fatty acids. More specifically, desired PUFAs can be expressed in seed. Methods of isolating seed oils are known in the art. Thus, in addition to providing a source for PUFAs, seed oil components may be manipulated through the expression of the elongase genes, as well as perhaps desaturase genes, in order to provide seed oils that can be added to nutritional compositions, pharmaceutical compositions, animal feeds and cosmetics. Once again, a vector which comprises a DNA sequence encoding the elongase operably linked to a promoter, will be introduced into the plant tissue or plant for a time and under conditions sufficient for expression of the elongase gene. The vector may also comprise one or more genes which encode other enzymes, for example,  $\Delta 4$ -desaturase,  $\Delta 5$ -desaturase,  $\Delta 6$ -

desaturase,  $\Delta 8$ -desaturase,  $\Delta 9$ -desaturase,  $\Delta 10$ -desaturase,  $\Delta 12$ -desaturase,  $\Delta 13$ -desaturase,  $\Delta 15$ -desaturase,  $\Delta 17$ -desaturase and/or  $\Delta 19$ -desaturase. The plant tissue or plant may produce the relevant substrate (e.g., DGLA, GLA, STA, AA, ADA, EPA, 20:4n-3, etc.) upon which the enzymes act or a vector encoding enzymes which produce such substrates may be introduced into the plant tissue, plant cell, plant, or host cell of interest. In addition, substrate may be sprayed on plant tissues expressing the appropriate enzymes. Using these various techniques, one may produce PUFAs (e.g., n-6 unsaturated fatty acids such as DGLA, AA or ADA, or n-3 fatty acids such as EPA or DHA) by use of a plant cell, plant tissue, plant, or host cell of interest. It should also be noted that the invention also encompasses a transgenic plant comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in, for example, the seeds of the transgenic plant.

The substrates which may be produced by the host cell either naturally or transgenically, as well as the enzymes which may be encoded by DNA sequences present in the vector, which is subsequently introduced into the host cell, are shown in Figure 1.

In view of the above, the present invention also encompasses a method of producing one of the elongase enzymes described above comprising the steps of: 1) isolating the desired nucleotide sequence of the elongase cDNA; 2) constructing a vector comprising said nucleotide sequence; and 3) introducing said vector into a host cell under time and conditions sufficient for the production of the elongase enzyme.

The present invention also encompasses a method of producing polyunsaturated fatty acids comprising exposing an acid to the elongase(s) produced as above such that the elongase

converts the acid to a polyunsaturated fatty acid. For example, when GLA is exposed to elongase, it is converted to DGLA. DGLA may then be exposed to  $\Delta 5$ -desaturase which converts the DGLA to AA. The AA may then be converted to EPA by use of  $\Delta 17$ -

5 desaturase which may be, in turn, converted to DHA by use of elongase and a  $\Delta 4$ -desaturase. Alternatively, elongase may be utilized to convert  $18:4n-3$  to  $20:4n-3$  which may be exposed to  $\Delta 5$ -desaturase and converted to EPA. Elongase may also be used to convert  $18:3n-3$  to  $20:3n-3$ , which may be, in turn, converted  
10 to  $20:4n-3$  by a  $\Delta 8$ -desaturase. Thus, elongase may be used in the production of polyunsaturated fatty acids which may be used, in turn, for particular beneficial purposes. (See Figure 1 for an illustration of the many critical roles elongase plays in several biosynthetic pathways.)

#### Uses of the Elongase Gene and Enzyme Encoded Thereby

As noted above, the isolated elongase cDNAs and the corresponding elongase enzymes (or purified polypeptides) encoded thereby have many uses. For example, each cDNA and  
20 corresponding enzyme may be used indirectly or directly in the production of polyunsaturated fatty acids, for example, DGLA, AA, ADA,  $20:4n-3$  or EPA. ("Directly" is meant to encompass the situation where the enzyme directly converts the acid to another acid, the latter of which is utilized in a composition (e.g.,  
25 the conversion of GLA to DGLA)). "Indirectly" is meant to encompass the situation where a fatty acid is converted to another fatty acid (i.e., a pathway intermediate) by elongase (e.g., GLA to DGLA) and then the latter fatty acid is converted to another fatty acid by use of a non-elongase enzyme (e.g.,  
30 DGLA to AA by  $\Delta 5$ -desaturase)). These polyunsaturated fatty acids (i.e., those produced either directly or indirectly by

activity of the elongase enzyme) may be added to, for example, nutritional compositions, pharmaceutical compositions, cosmetics, and animal feeds, all of which are encompassed by the present invention. These uses are described, in detail, below.

5

#### Nutritional Compositions

The present invention includes nutritional compositions. Such compositions, for purposes of the present invention, include any food or preparation for human consumption including for enteral or parenteral consumption, which when taken into the body (a) serve to nourish or build up tissues or supply energy and/or (b) maintain, restore or support adequate nutritional status or metabolic function.

15

The nutritional composition of the present invention comprises at least one oil or acid produced by use of at least one elongase enzyme, produced using the respective elongase gene, and may either be in a solid or liquid form. Additionally, the composition may include edible macronutrients, vitamins and minerals in amounts desired for a particular use. The amount of such ingredients will vary depending on whether the composition is intended for use with normal, healthy infants, children or adults having specialized needs such as those which accompany certain metabolic conditions (e.g., metabolic disorders).

25

Examples of macronutrients which may be added to the composition include but are not limited to edible fats, carbohydrates and proteins. Examples of such edible fats include but are not limited to coconut oil, soy oil, and mono- and diglycerides. Examples of such carbohydrates include but are not limited to glucose, edible lactose and hydrolyzed starch. Additionally, examples of proteins which may be

30

utilized in the nutritional composition of the invention include but are not limited to soy proteins, electrodialysed whey, electrodialysed skim milk, milk whey, or the hydrolysates of these proteins.

5       With respect to vitamins and minerals, the following may be added to the nutritional compositions of the present invention: calcium, phosphorus, potassium, sodium, chloride, magnesium, manganese, iron, copper, zinc, selenium, iodine, and Vitamins A, E, D, C, and the B complex. Other such vitamins and minerals may  
10       also be added.

      The components utilized in the nutritional compositions of the present invention will be of semi-purified or purified origin. By semi-purified or purified is meant a material which has been prepared by purification of a natural material or by  
15       synthesis.

      Examples of nutritional compositions of the present invention include but are not limited to infant formulas, dietary supplements, dietary substitutes, and rehydration compositions. Nutritional compositions of particular interest  
20       include but are not limited to those utilized for enteral and parenteral supplementation for infants, specialist infant formulae, supplements for the elderly, and supplements for those with gastrointestinal difficulties and/or malabsorption.

      The nutritional composition of the present invention may  
25       also be added to food even when supplementation of the diet is not required. For example, the composition may be added to food of any type including but not limited to margarines, modified butters, cheeses, milk, yogurt, chocolate, candy, snacks, salad oils, cooking oils, cooking fats, meats, fish and beverages.

30       In a preferred embodiment of the present invention, the nutritional composition is an enteral nutritional product, more preferably, an adult or pediatric enteral nutritional product.

This composition may be administered to adults or children experiencing stress or having specialized needs due to chronic or acute disease states. The composition may comprise, in addition to polyunsaturated fatty acids produced in accordance with the present invention, macronutrients, vitamins and minerals as described above. The macronutrients may be present in amounts equivalent to those present in human milk or on an energy basis, i.e., on a per calorie basis.

Methods for formulating liquid or solid enteral and parenteral nutritional formulas are well known in the art. (See also the Examples below.)

The enteral formula, for example, may be sterilized and subsequently utilized on a ready-to-feed (RTF) basis or stored in a concentrated liquid or powder. The powder can be prepared by spray drying the formula prepared as indicated above, and reconstituting it by rehydrating the concentrate. Adult and pediatric nutritional formulas are well known in the art and are commercially available (e.g., Similac®, Ensure®, Jevity® and Alimentum® from Ross Products Division, Abbott Laboratories, Columbus, Ohio). An oil or fatty acid produced in accordance with the present invention may be added to any of these formulas.

The energy density of the nutritional compositions of the present invention, when in liquid form, may range from about 0.6 Kcal to about 3 Kcal per ml. When in solid or powdered form, the nutritional supplements may contain from about 1.2 to more than 9 Kcals per gram, preferably about 3 to 7 Kcals per gm. In general, the osmolality of a liquid product should be less than 700 mOsm and, more preferably, less than 660 mOsm.

The nutritional formula may include macronutrients, vitamins, and minerals, as noted above, in addition to the PUFAs produced in accordance with the present invention. The presence



of these additional components helps the individual ingest the minimum daily requirements of these elements. In addition to the provision of PUFAs, it may also be desirable to add zinc, copper, folic acid and antioxidants to the composition. It is  
5 believed that these substance boost a stressed immune system and will therefore provide further benefits to the individual receiving the composition. A pharmaceutical composition may also be supplemented with these elements.

In a more preferred embodiment, the nutritional composition  
10 comprises, in addition to antioxidants and at least one PUFA, a source of carbohydrate wherein at least 5 weight % of the carbohydrate is indigestible oligosaccharide. In a more preferred embodiment, the nutritional composition additionally comprises protein, taurine, and carnitine.

15 As noted above, the PUFAs produced in accordance with the present invention, or derivatives thereof, may be added to a dietary substitute or supplement, particularly an infant formula, for patients undergoing intravenous feeding or for preventing or treating malnutrition or other conditions or  
20 disease states. As background, it should be noted that human breast milk has a fatty acid profile comprising from about 0.15% to about 0.36% as DHA, from about 0.03% to about 0.13% as EPA, from about 0.30% to about 0.88% as AA, from about 0.22% to about 0.67% as DGLA, and from about 0.27% to about 1.04% as GLA. Thus,  
25 fatty acids such as DGLA, AA, EPA and/or docosahexaenoic acid (DHA), produced in accordance with the present invention, can be used to alter, for example, the composition of infant formulas in order to better replicate the PUFA content of human breast milk or to alter the presence of PUFAs normally found in a non-  
30 human mammal's milk. In particular, a composition for use in a pharmacologic or food supplement, particularly a breast milk substitute or supplement, will preferably comprise one or more

of AA, DGLA and GLA. More preferably, the oil blend will comprise from about 0.3 to 30% AA, from about 0.2 to 30% DGLA, and/or from about 0.2 to about 30% GLA.

Parenteral nutritional compositions comprising from about 2  
5 to about 30 weight percent fatty acids calculated as triglycerides are encompassed by the present invention. The preferred composition has about 1 to about 25 weight percent of the total PUFA composition as GLA (U.S. Patent No. 5,196,198). Other vitamins, particularly fat-soluble vitamins such as  
10 vitamin A, D, E and L-carnitine can optionally be included. When desired, a preservative such as alpha-tocopherol may be added in an amount of about 0.1% by weight.

In addition, the ratios of AA, DGLA and GLA can be adapted for a particular given end use. When formulated as a breast  
15 milk supplement or substitute, a composition which comprises one or more of AA, DGLA and GLA will be provided in a ratio of about 1:19:30 to about 6:1:0.2, respectively. For example, the breast milk of animals can vary in ratios of AA:DGLA:GLA ranging from 1:19:30 to 6:1:0.2, which includes intermediate ratios which are  
20 preferably about 1:1:1, 1:2:1, 1:1:4. When produced together in a host cell, adjusting the rate and percent of conversion of a precursor substrate such as GLA and DGLA to AA can be used to precisely control the PUFA ratios. For example, a 5% to 10% conversion rate of DGLA to AA can be used to produce an AA to  
25 DGLA ratio of about 1:19, whereas a conversion rate of about 75% to 80% can be used to produce an AA to DGLA ratio of about 6:1. Therefore, whether in a cell culture system or in a host animal, regulating the timing, extent and specificity of elongase expression, as well as the expression of other desaturases, can  
30 be used to modulate PUFA levels and ratios. The PUFAs/acids produced in accordance with the present invention (e.g., AA and

DGLA) may then be combined with other PUFAs/acids (e.g., GLA) in the desired concentrations and ratios.

Additionally, PUFA produced in accordance with the present invention or host cells containing them may also be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal consumption.

#### Pharmaceutical Compositions

The present invention also encompasses a pharmaceutical composition comprising one or more of the fatty acids and/or resulting oils produced using at least one of the elongase cDNAs (i.e., MAELO, GLELO, HSELO1, or CELO), in accordance with the methods described herein. More specifically, such a pharmaceutical composition may comprise one or more of the acids and/or oils as well as a standard, well-known, non-toxic pharmaceutically acceptable carrier, adjuvant or vehicle such as, for example, phosphate buffered saline, water, ethanol, polyols, vegetable oils, a wetting agent or an emulsion such as a water/oil emulsion. The composition may be in either a liquid or solid form. For example, the composition may be in the form of a tablet, capsule, ingestible liquid or powder, injectible, or topical ointment or cream. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents, for example, sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening agents, flavoring agents and perfuming agents.

Suspensions, in addition to the active compounds, may comprise suspending agents such as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth or mixtures of these substances.

Solid dosage forms such as tablets and capsules can be prepared using techniques well known in the art. For example, PUFAs produced in accordance with the present invention can be tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders such as acacia, cornstarch or gelatin, disintegrating agents such as potato starch or alginic acid, and a lubricant such as stearic acid or magnesium stearate. Capsules can be prepared by incorporating these excipients into a gelatin capsule along with antioxidants and the relevant PUFA(s). The antioxidant and PUFA components should fit within the guidelines presented above.

For intravenous administration, the PUFAs produced in accordance with the present invention or derivatives thereof may be incorporated into commercial formulations such as Intralipids™. The typical normal adult plasma fatty acid profile comprises 6.64 to 9.46% of AA, 1.45 to 3.11% of DGLA, and 0.02 to 0.08% of GLA. These PUFAs or their metabolic precursors can be administered alone or in combination with other PUFAs in order to achieve a normal fatty acid profile in a patient. Where desired, the individual components of the formulations may be provided individually, in kit form, for single or multiple use. A typical dosage of a particular fatty acid is from 0.1 mg to 20 g (up to 100 g) daily and is preferably from 10 mg to 1, 2, 5 or 10 g daily.

Possible routes of administration of the pharmaceutical compositions of the present invention include, for example,

enteral (e.g., oral and rectal) and parenteral. For example, a liquid preparation may be administered, for example, orally or rectally. Additionally, a homogenous mixture can be completely dispersed in water, admixed under sterile conditions with  
5 physiologically acceptable diluents, preservatives, buffers or propellants in order to form a spray or inhalant.

The route of administration will, of course, depend upon the desired effect. For example, if the composition is being utilized to treat rough, dry, or aging skin, to treat injured or  
10 burned skin, or to treat skin or hair affected by a disease or condition, it may perhaps be applied topically.

The dosage of the composition to be administered to the patient may be determined by one of ordinary skill in the art and depends upon various factors such as weight of the patient,  
15 age of the patient, immune status of the patient, etc.

With respect to form, the composition may be, for example, a solution, a dispersion, a suspension, an emulsion or a sterile powder which is then reconstituted.

The present invention also includes the treatment of  
20 various disorders by use of the pharmaceutical and/or nutritional compositions described herein. In particular, the compositions of the present invention may be used to treat restenosis after angioplasty. Furthermore, symptoms of inflammation, rheumatoid arthritis, asthma and psoriasis may  
25 also be treated with the compositions of the invention. Evidence also indicates that PUFAs may be involved in calcium metabolism; thus, the compositions of the present invention may, perhaps, be utilized in the treatment or prevention of osteoporosis and of kidney or urinary tract stones.

30 Additionally, the compositions of the present invention may also be used in the treatment of cancer. Malignant cells have been shown to have altered fatty acid compositions. Addition of

fatty acids has been shown to slow their growth, cause cell death and increase their susceptibility to chemotherapeutic agents. Moreover, the compositions of the present invention may also be useful for treating cachexia associated with cancer.

5       The compositions of the present invention may also be used to treat diabetes (see U.S. Patent No. 4,826,877 and Horrobin et al., Am. J. Clin. Nutr. Vol. 57 (Suppl.) 732S-737S). Altered fatty acid metabolism and composition have been demonstrated in diabetic animals.

10       Furthermore, the compositions of the present invention, comprising PUFAs produced either directly or indirectly through the use of the elongase enzyme(s), may also be used in the treatment of eczema, in the reduction of blood pressure, and in the improvement of mathematics examination scores.

15       Additionally, the compositions of the present invention may be used in inhibition of platelet aggregation, induction of vasodilation, reduction in cholesterol levels, inhibition of proliferation of vessel wall smooth muscle and fibrous tissue (Brenner et al., Adv. Exp. Med. Biol. Vol. 83, p.85-101, 1976),  
20       reduction or prevention of gastrointestinal bleeding and other side effects of non-steroidal anti-inflammatory drugs (see U.S. Patent No. 4,666,701), prevention or treatment of endometriosis and premenstrual syndrome (see U.S. Patent No. 4,758,592), and treatment of myalgic encephalomyelitis and chronic fatigue after  
25       viral infections (see U.S. Patent No. 5,116,871).

      Further uses of the compositions of the present invention include use in the treatment of AIDS, multiple sclerosis, and inflammatory skin disorders, as well as for maintenance of general health.

30       Additionally, the composition of the present invention may be utilized for cosmetic purposes. It may be added to pre-

existing cosmetic compositions such that a mixture is formed or may be used as a sole composition.

#### Veterinary Applications

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It should be noted that the above-described pharmaceutical and nutritional compositions may be utilized in connection with animals (i.e., domestic or non-domestic), as well as humans, as animals experience many of the same needs and conditions as  
10 humans. For example, the oil or acids of the present invention may be utilized in animal feed supplements, animal feed substitutes, animal vitamins or in animal topical ointments.

The present invention may be illustrated by the use of the following non-limiting examples:

15

#### Example I

##### Determination of Codon Usage in *Mortierella alpina*

20 The 5' end of 1000 random cDNA clones were sequenced from *Mortierella alpina* cDNA library. The sequences were translated in six reading frames using GCG (Genetics Computer Group (Madison, Wisconsin)) with the FastA  
algorithm (Pearson and Lipman, Proc. Natl. Acad. Sci. USA  
25 85:2444-2448 (1988)) to search for similarity between a query sequence and a group of sequences of the same type (nucleic acid or protein), specifically with the  
Swissprot database (GeneBio, Geneva, Switzerland). Many of the clones were identified as a putative housekeeping  
30 gene based on protein sequence homology to known genes. Twenty-one M. alpina cDNA sequences which matched with known, housekeeping genes in the database were selected

(see Table 1 below). M. alpina codon bias table (see Table 2) was generated based on these 21 sequences as well as the full length M. alpina  $\Delta 5$ - (see Figure 18),  $\Delta 6$ -, and  $\Delta 12$ -desaturase sequences. Since the FastA alignment between the putative protein coded by the M. alpina cDNA sequence and the known protein sequence was weak in some areas, only the codons from areas of strong homology were used.

Table 1

Clone #	Match	# of bp	# of aa
193	Elongation factor 1-alpha	426	142
143	60S ribosomal protein L17	417	139
235	Actin I	360	120
299	40S ribosomal protein YS11	387	129
390	Ras-related protein rab-1a	342	114
65	40S ribosomal protein RP10	366	122
289	Ubiquitin-conjugating enzyme E2-16 KD	294	98
151	Ubiquinol-cytochrome C reductase	375	125
80	Initiation factor 5A-2	183	61
33	60S ribosomal protein L15	252	84



Table 1 (continued)

Clone #	Match	# of bp	# of aa
132	60S ribosomal protein L3-2	300	100
198	Histone H3	285	95
286	6-phosphogluconate dehydrogenase, decarboxylating	363	121
283	40S ribosomal protein S22	261	87
127	Elongation factor 2	231	77
197	Actin, gamma	252	84
496	40S ribosomal protein S16	270	90
336	Histone H4	219	73
262	Ubiquitin	228	76
188	Guanine nucleotide-binding protein beta subunit-like protein	213	71
81	Ubiquitin	228	76
21	TOTAL	6252	2084

5

Table 2

	Amino acid	Codon Bias	% used	Amino acid	Codon Bias	% used
10	Ala	GCC	63%	Lys	AAG	96%
	Arg	CGC	50%	Met	ATG	100%
	Asn	AAC	97%	Phe	TTC	78%
	Asp	GAC	65%	Pro	CCC	68%
15	Cys	TGC	87%	Ser	TCC	46%
	Gln	CAG	78%	Thr	ACC	78%
	Glu	GAG	85%	Trp	TGG	100%
	Gly	GGT	47%	Tyr	TAC	95%
	His	CAC	91%	Val	GTC	72%
20	Ile	ATC	72%	Stop	TAA	50%
	Leu	CTC	49%			

Example IICloning of a Full-length Elongase-like cDNA from *M. alpina*

5        The  $\beta$ -ketoacyl-coenzyme A synthase (KCS) from jojoba and the *Saccharomyces cerevisiae* elongase (ELO2) were aligned to determine an area of amino acid homology (see Figure 2). The codon bias was applied to the area of sequence corresponding to the homologous amino acids  
10        between the two elongases, and primers were designed based on this biased sequence (see Figure 3). The cDNA was excised from the M11 *M. alpina* cDNA library (Knutzon et al., J. Biol. Chem. 273:29360-29366 (1998)), which contains approximately  $6 \times 10^5$  clones with an average  
15        insert size of 1.1 Kb. The excised cDNA was amplified with internal primer RO339 (5' -TTG GAG AGG AGG AAG CGA CCA CCG AAG ATG ATG- 3') and a vector forward primer RO317 (5' - CAC ACA GGA AAC AGC TAT GAC CAT GAT TAC G - 3'). Polymerase Chain Reaction (PCR) was carried out in  
20        a 100  $\mu$ l volume containing: 300 ng of excised *M. alpina* cDNA library, 50 pmole each primer, 10  $\mu$ l of 10X buffer, 1  $\mu$ l 10 mM PCR Nucleotide Mix (Boehringer Mannheim Corp., Indianapolis, IN) and 1.0 U of Taq Polymerase. Thermocycler conditions in Perkin Elmer 9600 (Norwalk, CT) were as follows: 94°C for 2 mins., then 30 cycles of  
25        94°C for 1 min., 58°C for 2 mins., and 72°C for 3 mins. PCR was followed by an additional extension at 72°C for 7 minutes.

30        The PCR amplified product was run on a gel, an amplified fragment of approximately 360 bp was gel purified, and the isolated fragment was directly sequenced using ABI 373A DNA Sequencer (Perkin Elmer,

Foster City, CA). The sequence analysis package of GCG was used to compare the obtained sequence with known sequences. The sequence was translated in all six reading frames in the GCG Analysis Program using the FastA algorithm (Pearson and Lipman, supra). The Swissprot database (GeneBio, Geneva, Switzerland) of proteins was searched. This translated cDNA fragment was identified as a part of a putative elongase based on the homology of the putative protein sequence to the S. cerevisiae ELO2 (GNS1), having 41.3% identity in 63 amino acids.

New primers were designed based on the putative elongase sequence and the vector, pZL1 (Life Technologies, Inc., Gaithersburg, MD) sequence used to construct M. alpina cDNA library. The M. alpina excised cDNA library was PCR amplified again using primers RO350 (5' -CAT CTC ATG GAT CCG CCA TGG CCG CCG CAA TCT TG- 3'), which has an added *Bam*HI restriction site (underlined), and the vector reverse primer RO352 (5' -ACG CGT ACG TAA AGC TTG- 3') to isolate the full length M. alpina elongase cDNA, using previously described conditions. The termini of the approximately 1.5 Kb PCR amplified fragment was filled-in with T4 DNA polymerase (Boehringer Mannheim Corp., Indianapolis, IN) to create blunt ends and cloned into the pCR-blunt vector (Invitrogen Corp., Carlsbad, CA). This resulted in two clones, pRAE-1 and pRAE-2 (see Figure 4A). (Plasmid DNA pRAE-2 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, on August 28, 1998, under the terms of the Budapest Treaty, and was accorded deposit number ATCC 203166.) The elongase cDNAs from these vectors were cut out as an *Eco*RI fragment and cloned into the *Eco*RI digested pYX242

(Novagen, Madison, WI) vector. The clones pRAE-5 and pRAE-6 (see Figure 4B) have the elongase cDNAs from pRAE-1 and pRAE-2, respectively. (Plasmid DNA pRAE-5 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, on August 28, 1998, under the terms of the Budapest Treaty, and was accorded deposit number ATCC 203167.) The sequencing of pRAE-5 and pRAE-6 revealed that 5' untranslated region of the elongase gene in pRAE-5 is 16 bp shorter than that in pRAE-6 (see Figure 5). The complete *M. alpina* elongase cDNA sequence, designated MAELO was obtained from pRAE-2 (see Figure 6). Figure 7 is the amino acid sequence obtained from the translation of MAELO. The Swissprot database (GeneBio, Geneva, Switzerland) was searched again, as previously described, with the translated MAELO: MAELO has 44.3% identity in 317 amino acids with *S. cerevisiae* GNS1(ELO2) and 44.7% identity in 318 amino acids with *S. cerevisiae* SUR4(ELO3). The FastA alignment among the three elongases is shown in Figure 8. At the nucleotide level (see Figure 9), MAELO has 57.4% identity in 549 bp overlap with *S. cerevisiae* GNS1(ELO2) (GenBank Accession # S78624). However, the identity between the complete MAELO gene of 954 bp and *S. cerevisiae* GNS1(ELO2) is 33.0%.

### Example III

#### Expression of *M. alpina* Elongase cDNA in Baker's Yeast

The constructs pRAE-5, and pRAE-6 were transformed into *S. cerevisiae* 334 (Hoveland et al., *Gene* 83:57-64 (1989)) and screened for elongase activity. The plasmid

pCGN7875 (Calgene LLC, Davis, CA) containing jojoba KCS gene in pYES2 vector (Invitrogen Corp., Carlsbad, CA) was used as a positive control. The substrate used to detect elongase activity in *M. alpina* elongase (MAELO) was GLA and that in jojoba KCS was oleic acid (OA). The negative control strain was *S. cerevisiae* 334 containing pYX242 vector. The cultures were grown for 40-48 hours at 25°C, in selective media (Ausubel et al., Short Protocols in Molecular Biology, Ch. 13, p. 3-5 (1992)), in the presence of a particular substrate. The expression of the jojoba KCS gene cloned in pYES2 was under the control of GAL1 promoter, while the promoter in pYX242 is TP1, which is constitutive. Hence, the 334(pCGN7875) and 334(pYES2) cultures were induced with galactose. The GC-FAME analysis of the lipid fraction of each cell pellet was performed as previously described (Knutzon et al., supra).

The elongase activity results from different experiments are provided in Figure 10A and 10B. The jojoba KCS elongates long chain monounsaturated fatty acids 18:1n-9 to 20:1n-9. The amino acid homology between the *M. alpina* elongase (MAELO) and the *S. cerevisiae* ELO2 and ELO3 suggested that the proteins encoded by these genes may have similar substrate specificity. The activity of the *M. alpina* elongase, elongation (MAELO) of long chain monounsaturated and saturated fatty acids, is seen in the conversion of 18:1n-9 to 20:1n-9 and also in the synthesis of 24:0. The control strain, 334(pYX242) has very little or no detectable amount of 20:1 and 24:0 (see Figure 10A). *M. alpina* elongase (MAELO) also acts on at least one PUFA, converting 18:3n-6 (GLA) to 20:3n-6 (DGLA). The percentage

of the 20:3n-6 in total lipid is higher in the strain 334(pRAE-5) and 334(pRAE-6) with the *M. alpina* elongase (MAELO) cDNA when compared to that in the control 334(pYX242). The percentages of 20:3n-6 produced were  
5 0.092% for 334(pYX242) vs. 0.324% for 334(pRAE-5) and 0.269% for 334(pRAE-6) (shown in parenthesis in Figures 10A and 10B). This difference in the fatty acid profile is also seen in the total amount of 20:3n-6 produced. Only 0.226  $\mu$ g of 20:3n-6 was produced by 334(pYX242)  
10 while 334(pRAE-5) and 334(pRAE-6) produced 2.504  $\mu$ g of 20:3n-6 and 1.006  $\mu$ g of 20:3n-6, respectively. Also, when no substrate is added, the level of 20:3n-6 is not detectable.

Once 20:3n-6 is generated by the *M. alpina* elongase  
15 (MAELO), the  $\Delta 5$ -desaturase can convert it to AA in the desired expression system. To test this hypothesis, the constructs pRAE-5 and pCGR-4 (a  $\Delta 5$ -desaturase containing plasmid) were co-transformed into *S. cerevisiae* 334 and screened for AA production. The substrate used was 25  $\mu$ M  
20 GLA (18:3n-6). If the *M. alpina* elongase (MAELO) is active in yeast, then the substrate will be converted to DGLA(20:3n-6), which the  $\Delta 5$ -desaturase will convert to AA(20:4n-6). The results in Figure 11 confirm the production of AA and therefore, the activity of the *M.*  
25 *alpina* elongase (MAELO).

The expression of  $\Delta 5$ -,  $\Delta 6$ -, and  $\Delta 12$ -desaturases, in yeast, along with the elongase, should result in the production of AA (see Figure 1) without the need for an exogenous supply of fatty acids.

#### Example IV

##### A Comparison of the Expression of *M. alpina* Elongase cDNA MAELO and *S. cerevisiae* Elongase ELO2 in Baker's Yeast

5       The ELO2 gene encoding for the yeast elongase was cloned from an *S. cerevisiae* genomic library (Origene, Rockville, MD) using the primers RO514 (5' -GGC TAT GGA TCC ATG AAT TCA CTC GTT ACT CAA TAT G-3') and RO515 (5' -CCT GCC AAG CTT TTA CCT TTT TCT TCT GTG TTG AG-3') incorporating the restriction sites (underlined) *Bam*HI and *Hind*III (respectively). The ELO2 gene was cloned into the vector pYX242 at the *Bam*HI and *Hind*III sites, designated pRELO, transformed into the *S. cerevisiae* host 334 (Hoveland et al., *supra*) and screened for PUFA elongase activity. The vector plasmid was used as a negative control and 334(pRAE-5) was grown to compare the PUFA elongase activity. The cultures were grown as previously described with no galactose in the media and 25  $\mu$ M GLA added as a substrate. Figure 12 shows that amount of 20:3n-6 or DGLA produced (elongated from 18:3n-6 or GLA) by 334(pRAE-5) was approximately 4 times the negative control containing the unaltered vector pYX242, while the two individual clones 334(pRELO-1) and 334(pRELO-2) were only twice the negative control. Additionally, when DGLA produced is expressed as a percent of the total lipids (shown in parenthesis, Figure 12), the clones 334(pRELO-1) and 334 (pRELO-2) produced 0.153% and 0.2% DGLA respectively, while 334(pYX242) produced 0.185% DGLA. Hence all these strains produced comparable percentages of DGLA. The strain 334(pRAE-5), however, produced 0.279% DGLA, an increase of 50.8% over 334(pYX242) (negative control). These data show that the *S. cerevisiae* elongase gene ELO2, even when overexpressed in yeast, does not elongate GLA to DGLA effectively. The *M. alpina* PUFA elongase activity is specific

for this conversion as evidenced by the higher amount of DGLA produced compared to the control, 334(pYX242).

#### Example V

##### Identification of Elongases from Other Sources Using MAELO

The TFastA algorithm (Pearson and Lipman, supra) is used to search for similarity between a query peptide sequence and the database DNA sequence translated in each of the six reading frames. Translated MAELO was used as the query for a TFastA search in GCG with the GenEMBL database (6/98) from GCG to identify other potential elongase sequences based on their amino acid similarity comparisons to translated MAELO. For example, in Figures 13 and 14, two alignments are shown between translations of two different C. elegans sequences from chromosome III and MAELO. C. elegans DNA sequence (GenBank accession # Z68749) was annotated denoting similarity with GNS1 (ELO2), while the additional C. elegans DNA sequence (GenBank accession # U61954) was noted as similar to both GNS1 and SUR4 (ELO3). These are spliced DNA fragments in which the introns have been removed from the genomic sequence, and the exons assembled and translated. The amount of amino acid identity between the putative PUFA elongases from C. elegans and translated MAELO are around 30%. This would point towards a common function in the fatty acid metabolism, e. g., a PUFA elongase. Figure 15 is another example of a translated C. elegans sequence (GenBank accession # AF003134) from chromosome III. The DNA sequence was identified that had DNA homology to the S. cerevisiae ELO2. Further inspection of this DNA sequence and its amino acid translation determined that there was homology to translated MAELO. C. elegans, therefore, may contain a PUFA elongase.



Figure 16 shows the alignments of translated DNA sequences from mouse and human, respectively, with translated MAELO. The mouse sequence CIG30, GenBank accession # U97107, was isolated from brown adipose tissue and reported as being "similar to yeast SUR4 protein". As shown in Figure 16, amino acids numbered 130 to 152 in the U97107 translation contain a high degree of similarity to the translated MAELO. The human sequence, GenBank accession # AC004050, from chromosome 4 was from an HTGS (High Throughput Genome Sequence). There were no annotations contained with this sequence. However, translated AC004050 had 28.7% identity in 150 amino acids with translated MAELO. This gene fragment could be a fragment of a human PUFA elongase based on its amino acid similarity to translated MAELO.

Figure 17 shows the amino acid alignment of translated MAELO and a mammalian sequence (GenBank accession # I05465, PCT# WO 88/ 07577) which claims that the protein derived from expression of this sequence is a glycosylation inhibition factor. The amino acid identities between the two proteins, signifying that there could be related function, such as PUFA elongase activity.

These examples of other translated DNA sequences and their homology to the translated MAELO illustrate that any of the above examples could potentially be a PUFA elongase. These examples are not inclusive of all the possible elongases. However, use of MAELO or its amino acid translation as a query for database searches can identify other genes which have PUFA elongase activities.

#### Example VI

#### M. alpina cDNA Library Screening Using A Plaque Hybridization Method

In an effort to isolate additional PUFA elongase genes from M. alpina, a conventional plaque hybridization method was used to screen an M. alpina cDNA library made in a lambda vector. The DNA probe was generated based on MAELO nucleotide sequence and was used to screen the M7+8 M. alpina cDNA library made in a  $\lambda$ Ziplox vector (Knutzon et al., J. Biol. Chem. 273:29360-29366 (1998)).

To make the DNA probe for screening the library, the MAELO cDNA was digested with *NspI* and *PvuI* restriction endonucleases. Three small DNA fragments, with an average size of approximately 300 bp, were produced and used as probes. The rationale for using a mixture of fragmented MAELO cDNA was based on the assumption that there might be a common region or domain in the amino acid sequence which is conserved among various PUFA elongases present in M. alpina. Using MAELO DNA probes, the cDNA library was screened by a plaque hybridization technique according to standard protocol (Sambrook et al., Molecular Cloning, 2<sup>nd</sup> Ed., Cold Spring Harbor, 1989).

Briefly, 50,000 primary clones were plated and transferred to nylon membranes. The membranes were denatured and hybridized with alpha <sup>32</sup>P-dCTP-labelled MAELO DNA probes overnight in the hybridization buffer which contained 20% formamide, 0.2% PVP, BSA, Ficoll, 0.1% SDS and 0.5 M NaCl. The filters were washed with 0.5X SSC at 37 °C and exposed to X-ray film for autoradiography. This procedure was repeated three times. Four clones (designated as F1, F2, F3, and F4) which hybridized repeatedly were picked and suspended in SM buffer (Sambrook et al., supra) containing 7% DMSO.

The largest open reading frame of each candidate was subcloned into yeast expression vector pYX242 (Novagen, Inc., Madison, Wisconsin). The cDNA clones F1 and F3 were subcloned into pYX242 at the *EcoRI* site while F2 and F4 were subcloned at

*NcoI/HindIII* sites. The recombinant pYX242 containing each candidate was transformed into SC334 (Hoveland et al., supra) for expression in yeast. To determine the elongase activity, as well as substrate specificity, SC334 containing each cDNA clone was grown in minimal media lacking leucine in the presence of 25  $\mu$ M of GLA substrate as described in Example III. The fatty acid analysis was performed as described in Knutzon et al. (J. Biol. Chem. 273:29360-29366 (1998)). The results indicated that none of these four cDNA clones showed any significant activity in converting GLA to DGLA. Thus, the hybridization approach appeared to be unsuccessful in identifying additional PUFA elongases.

#### Example VII

##### Construction of Direct cDNA Expression Library of *M. alpina* in Yeast

To identify PUFA elongase genes other than MAELO, a different approach was taken to screen the *M. alpina* cDNA library. In particular, since Baker's yeast is incapable of producing long chain PUFAs due to the absence of respective desaturases and elongases, an attempt was made to construct an expression cDNA library of *M. alpina* in *Saccharomyces cerevisiae*. The vector pYES2 (Novagen, Inc., Madison, Wisconsin), containing the GAL1 promoter, was chosen for the expression of cDNA library in *S. cerevisiae*.

The conventional way by which a cDNA library is made (i.e. transformation of cDNA/vector ligated DNA mixture into host cells) is difficult in yeast because the transformation efficiency by direct electroporation of ligated DNA mix is very low compared to the efficiency of purified supercoiled plasmid DNA. However, the major advantage of this method is to avoid

amplification of primary clones which happens when the library is made in E. coli as an intermediate. Due to the limitation in the number of colonies to be screened, it was decided to first optimize the efficiency of transformation in different S. cerevisiae strains using cDNA/vector ligated mix. The best results were obtained with a yield of  $4-5 \times 10^5$  transformants per  $\mu\text{g}$  of ligated DNA in S. cerevisiae strain SC334 (Hoveland et al., supra).

To make a direct M. alpina cDNA expression library in yeast total RNA was isolated from the fungus. M. alpina fungus (ATCC # 32221) was plated onto cornmeal agar (Difco Laboratories, Detroit, MI) and grown at room temperature for 3-4 days. Once fungus growth was visible, it was inoculated into 50 ml of potato dextrose broth and shaken at room temperature very slowly to formulate spores. Once spores were visible, the 50 ml culture was inoculated into a 1 liter culture of potato dextrose, and spores were grown for 72 hours. After filtering through sterile gauze, the cells were immediately frozen into liquid nitrogen for future RNA extraction. Total RNA was prepared from 36 g of cell pellet using the hot phenol/LiCl extraction method (Sambrook et al., supra). The cell pellets were homogenized in a 10 mM EDTA, 1% SDS and 200 mM sodium acetate, pH 4.8 solution. Phenol and chloroform were added to the homogenates, and the aqueous layer was extracted. The aqueous layer was back extracted one more time with phenol and chloroform. Then an equal volume of 4 M lithium chloride was added. The samples were ethanol precipitated on ice for 3 hours, and pellets were obtained by centrifugation. The RNA pellets were washed with 70% ethanol and resuspended in DEPC treated water. Total RNA was quantitated by spectrophotometry and visualized by agarose gel electrophoresis to confirm the

presence of 28S and 18S ribosomal bands. Approximately, 15 mg of total RNA were obtained from 36 gram of cell pellet.

The library was constructed according to the standard protocol (Sambrook et. al., Molecular Cloning, 2<sup>nd</sup> Ed., Cold Spring Harbor, 1989). Messenger RNA was prepared from the total RNA using oligo dT cellulose affinity purification. Messenger RNA was reverse transcribed with oligo dT primer containing a XhoI restriction site using AMV reverse transcriptase. Following first strand cDNA synthesis, the second strand of cDNA was synthesized by adding E. coli DNA polymerase, E. coli DNA ligase and RNase H.

The EcoRI adaptor was ligated into the blunt-ended cDNA by T4 DNA ligase. The cDNA sample was kinased using T4 polynucleotide kinase and digested with XhoI, diluted with column buffer and passed through a Sephacryl S-400 column. The DNA samples were eluted by high salt buffer. Samples containing DNA from 400-5,000 bps were pooled and used for ligation into a pYES2 vector (Invitrogen Corp., Carlsbad, CA). The cDNA was ligated into the EcoRI/XhoI digested pYES2 vector using T4 DNA ligase. A large scale ligation reaction was carried out since a large amount of the ligated DNA (2-3  $\mu$ g) is required in direct transformation of yeast.

To transform yeast cells directly with the cDNA/pYES2 ligated mixture, competent SC334 cells were prepared using the LiAc TRAFO method (Gietz et. al., Mol. Cell. Biol, 5: 255-269, 1995). Briefly, fresh culture of SC334 from the plate was inoculated into 50 ml YPD medium. The culture was grown at 30 °C with shaking until the OD at 600 had reached 1.0. Thirty ml of this starter was inoculated into 300 ml of YPD liquid medium and incubated with shaking until the cell number of the culture reached ~ 3-5 x 10<sup>6</sup> cell/ml (approximately 3-4 h). The cells were harvested and washed with sterile water. The entire cell

pellet was resuspended in 1.5 ml of freshly prepared 1X TE/LiAc (0.1M LiAc). These cells were used immediately for the transformations.

Seven hundred and fifty microliters of competent SC334 cells were aliquoted into 15 ml falcon tubes. Approximately 2 ug of cDNA/pYES2 ligated DNA were added to the cells along with carrier DNA and mixed gently. Three milliliters of sterile 40% PEG/LiAc was added to the cells and mixed gently but thoroughly. The cells were incubated at 30 °C for 30 min with shaking and subsequently given heat shock at 42 °C for 15 min. The cells were cooled, pelleted, and resuspended in 5 ml of 1X TE. A 100 ul aliquot of the above cells was plated onto fifty 150 mm selective agar plates lacking uracil (Ausubel et al., supra) and incubated at 30 °C for 3 days. A total of  $8 \times 10^5$  primary clones were obtained. Five colonies were pooled in 1 ml minimal media lacking uracil (Ausubel et al., supra) and glycerol added to prepare stocks. A total of 5,000 pools were made for screening.

#### Example VIII

##### MAD (M. alpina Direct) Screening in Yeast

The quality of the library was analyzed by determining the average size of the cDNAs in the library. Since the screening of the library was based on the expression of the cDNA, it was important to determine the average size of the cDNA present in the library. The expression library containing the longest cDNAs would be the best appropriate choice to isolate full-length cDNAs of interest. To this end, randomly selected pools were plated onto selective agar plates, as described in Example VII, to obtain individual colonies. Forty different yeast colonies were randomly picked, and each colony was inoculated into 5 ml of selective liquid medium lacking uracil (as

described in Example VII) and grown, while shaking, for 24 hours at 30 °C. Plasmid DNA was extracted from these colonies by the bead beating method (Hoffman et al., Gene 57:267 (1987)) adapted as follows:

5 Pellets from 5 ml of culture were lysed in 0.5 ml of a 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA and 0.1% SDS solution. Sterile 0.5 mm glass beads of equal volume were added and manually vortexed for 3 minutes. Two hundred microliters of the same buffer were added, and the mixture was vortexed for an  
10 additional minute. The samples were centrifuged on high for 2 minutes, and cytoplasmic extract was then transferred to a fresh tube. An equal volume of phenol/CHCl<sub>3</sub> was added to the sample, vortexed and centrifuged again for 2 minutes. The aqueous layer was re-extracted twice and precipitated with 0.3 M sodium  
15 acetate and approximately 2.5 volumes of ethanol for 30 minutes at -20 °C. The precipitates were washed with 70% ethanol and resuspended in water. To eliminate RNA and any protein contamination, the plasmid DNAs isolated from 40 different samples were further purified using the QIAprep Spin Miniprep  
20 Kit according to the manufacturer's protocol (Qiagen Inc., Valencia, CA). The plasmid DNA samples were then restricted with *EcoRI* and *XhoI* restriction endonucleases to release the cDNA fragment, and the digest was analyzed on 1% agarose gel. The results indicated that the majority of the cDNAs of the  
25 direct library varied in length from 0.8 Kb to 1.5 Kb.

To screen the library, the glycerol stocks were thawed and approximately 0.5 ml was added to 5 ml of liquid selective media lacking uracil (Ausubel et al., supra) and grown at 30°C for 24 hours. The culture was then transferred into 50 ml of liquid  
30 selective medium lacking uracil with 2% galactose and 25 µM GLA (substrate for the elongase enzyme) for 24 hours at 25 °C with shaking. The GC-FAME analysis of the lipid content in the cell

pellet of each induced culture was performed as previously described (Knutzon et al., supra). The MAELO (pRAE-5 in pYX242 grown in selective media lacking leucine) was used as a positive control in each batch run. MAELO had consistently been able to  
5 convert 1.5% of GLA to DGLA (see Example III).

### Example IX

#### Identification of a cDNA Encoding a Potential PUFA Elongase

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After screening and analyzing approximately 750 individual pools by GC-FAME analysis, as described in Example VIII, one pool of five colonies (i.e., MAD708) appeared to have significant enzymatic activity in converting GLA to DGLA. This  
15 activity was found to be approximately 5 fold higher than the M. alpina elongase activity (MAELO) in terms of DGLA/GLA ratio (Figure 19). This pool was tested again under identical assay conditions to confirm the initial findings. The repeat experiment showed 9.5% conversion of GLA to DGLA and was again  
20 around 5 fold higher than M. alpina elongase activity (MAELO). These results strongly indicated that the MAD708 pool contained an elongase candidate which was specific for GLA as substrate. Since MAD708 was a pool of five different clones, it was necessary to isolate the individual cDNA clone which encoded for  
25 elongase activity from this pool. To do this, the original MAD708 glycerol stock was plated onto a selective media agar plate lacking uracil (Ausubel et al., supra). Thirty individual colonies were picked and grown in liquid selective medium, lacking uracil with 2% galactose, as previously described in  
30 Example VIII, in the presence of GLA. The cell pellet obtained from each culture was then subjected to fatty GC-FAME analysis (Knutzon et al., supra) along with a positive control of 334



(pRAE-5) (MAELO in PYX242). The fatty acid analysis from the 30 individual clones from the MAD708 expression pool in yeast revealed that 5 of the 30 clones showed elongase activity in converting GLA to DGLA. The fatty acid profiles of the active clones MAD708-2, MAD708-10, MAD708-18, MAD708-19 and MAD708-30 are shown in Figure 20. As shown in this Figure, MAD708-2, 10, and 30 produced the most DGLA, approximately 25 fold more than MAELO (pRAE-5). These 3 converted in the range of 41% to 49% of GLA to DGLA. Other clones, MAD708-18 and MAD708-19, converted 8% and 21% of GLA to DGLA, respectively. All MAD708 clones converted a higher percentage of GLA to DGLA with respect to MAELO encoded elongase (3.4%).

#### Example X

##### Characterization of cDNAs Encoding Elongase

Plasmid DNA was extracted from SC334 yeast clones (MAD708 pool) that showed significant GLA specific elongase activity by the bead beating method, as described in Example VIII. To determine the size of the cDNA insert, PCR was performed using each plasmid DNA obtained from positive elongase clones as a template. The forward primer RO541 (5'- GAC TAC TAG CAG CTG TAA TAC -3') and the reverse primer RO540 (5'- GTG AAT GTA AGC GTG ACA TAA -3') are in the multicloning site of the pYES2 vector and were used to amplify the cDNA insert within the *EcoRI* and *XhoI* sites. PCR reaction was performed in a 50  $\mu$ l volume containing 4  $\mu$ l of plasmid DNA, 50 pmole of each primer, 5  $\mu$ l of 10X buffer, 1  $\mu$ l 10  $\mu$ M PCR Nucleotide Mix (Boehringer Mannheim Corp., Indianapolis, IN) and 0.5  $\mu$ l of High Five Taq polymerase (Boehringer Mannheim, Indianapolis, IN). The amplification was carried out as follows: 2 mins. denaturation at 94 °C, then 94

°C for 1 min, 55 °C for 2 mins., and 72 °C for 3 mins. for 30 cycles, and 7 mins. extension at 72 °C at the end of the amplification. Analysis of PCR amplified products on a 1% agarose gel showed the sizes of the elongase cDNAs to be around  
5 1.0 - 1.2 Kb. The plasmid DNAs, containing the potential elongase cDNAs, were designated as pRPB2, pRPB10, pRPB18, pRPB19, and pRPB30. Since the cDNA library was made in the pYES2 vector at the *EcoRI* and *XhoI* sites, the size of the cDNA present in each plasmid was further confirmed by digesting the  
10 above plasmids with *EcoRI* and *XhoI*.

The plasmid DNAs isolated from yeast were re-amplified in *E. coli* for long-term storage of the cDNA clones as well as for DNA sequencing. *E. coli* TOP10 (Invitrogen Corp., Carlsbad, CA) cells were transformed with the pRPB recombinant plasmids  
15 according to the manufacturer's protocol. The transformants obtained from each plasmid DNA were inoculated into LB containing ampicillin (50 µg/ml) and grown overnight at 37 °C with shaking. Plasmid DNAs were isolated from these cultures by using QIAprep Spin Miniprep (Qiagen Inc., Valencia, CA)  
20 according to the manufacturer's protocol. The purified plasmid DNAs were then used for sequencing from both 5' and 3' ends. The DNA sequencing was performed by using a 373A Stretch ABI automated DNA sequencer (Perkin Elmer, Foster City, CA) according to the manufacturer's protocol. Primers used for  
25 sequencing were the forward primer RO541 (5'- GAT TAC TAG CAG CTG TAA TAC -3') and the reverse primer RO540 (5'- GTG AAT GTA AGC GTG ACA TAA -3') contained in the multicloning sites of the pYES2 vector. The obtained nucleotide sequences were transferred to Sequencher software program (Gene Codes  
30 Corporation, Ann Arbor, MI) for analysis. The DNA sequence analysis revealed that all five elongase cDNAs contained the identical nucleotide sequence with a common overlap of 301

nucleotides. Each DNA sequence contains a putative start site at the beginning of the 5' end and a stop codon with poly A tail at the end of the 3' site. To further confirm the DNA sequence, internal forward primers RO728 (5'- GAG ACT TTG AGC GGT TCG -3') and RO730 (5'- TCT CTG CTG CGT TGA ACT CG -3'), along with reverse primers RO729 (5'- AAA GCT CTT GAC CTC GAA C -3') and RO731 (5'- AAC TTG ATG AAC GAC ACG TG -3') were designed within the cDNA, and used for sequencing of pRPB2, since this candidate possessed the highest elongase activity.

The entire nucleotide sequence was analyzed by the Sequencer program (Figure 21), and the longest open reading frame deduced from the entire cDNA sequence in pRPB2 appeared to be 957 bp in length (Figure 22). The deduced open reading frame was then translated into the corresponding amino acid sequence, and the predicted sequence is shown in Figure 23. The elongase encoded by the cDNA (pRPB2) identified from *M. alpina* appears to be a 318 amino acid long protein which is nearly identical in size with translated MAELO. This new elongase cDNA was designated as "GLELO" and its encoded protein has been named "GLA elongase".

Plasmid DNA pRPB2 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 on July 22, 1999 under the terms of the Budapest Treaty. It was accorded ATCC Deposit # PTA-402.

#### Example XI

##### Biochemical Characterization of GLA Elongase (GLELO)

##### A. Confirmation of GLA Elongase Activity

To further confirm the activity of the GLA elongase encoded by the pRPB2 recombinant plasmid, elongase activity screening was repeated on the yeast clone SC334 containing pRPB2 plasmid. This experiment was also conducted to assure consistent lipid extraction and to detect the activity of GLA elongase by

averaging four independent experiments. The S. cerevisiae 334 glycerol stock containing pRPB2 was plated onto minimal media agar plates lacking uracil. Individual colonies were randomly picked and grown in minimal medium lacking uracil, as described in Example VIII. The four independent cultures were combined, and a 5 ml aliquot was used as an inoculum for four separate 50 ml cultures. The cultures were then grown in the presence of GLA and were subjected to fatty acid analysis along with a negative control of S. cerevisiae 334 containing pYES2, as described in Example VIII. The average elongase activity from four independent cultures of 334(pRPB2) with 25  $\mu$ M GLA is shown in Figure 24. The GLA elongase activity of each of the four independent samples of 334(pRPB2) appeared to be consistent with an average conversion of 62% GLA to DGLA.

#### B. Determination of GLELO Substrate Specificity for GLA Elongase

To analyze the substrate specificity of the GLA elongase, the culture of 334(pRPB2) was tested with different fatty acid substrates besides GLA (e.g., SA(18:0), OA(18:1), LA(18:2n-6), AA(20:4n-6), ADA(22:4n-6), ALA(18:3n-3), STA(18:4n-3), and EPA(20:5n-3)). Under identical assay conditions, the only other substrate utilized by the elongase enzyme was STA, a fatty acid from the n-3 pathway. GLA elongase was able to convert 73% of STA to 20:4n-3 (Figure 25). From these experiments, it can be concluded that the GLA elongase has substrate specificity for both GLA and STA, indicating that it possesses elongase activity along both the n-6 and n-3 pathways.

#### C. Co-expression of Fungal GLELO and $\Delta$ 5-Desaturase Gene in Yeast

Once DGLA (20:3n-6) is produced by the GLA elongase, the  $\Delta$ 5-desaturase can convert it to AA (20:4n-6) in a desired co-expression system. This scheme, as depicted in Figure 1, can be

tested by co-transforming S. cerevisiae 334 with plasmids pRPB2 and pRPE31 (the recombinant plasmid pYX242 containing a  $\Delta 5$ -desaturase cDNA (Figure 18) cloned at the EcoRI site. The co-transformed yeast cultures were supplemented with 25 $\mu$ M GLA and analyzed for AA synthesis. If both elongase and  $\Delta 5$ -desaturase enzymes are expressed, the GLA substrate will be converted to DGLA, which will then be converted to AA. The results in Figure 26A indicate that the sequential action of GLA elongase and  $\Delta 5$ -desaturase on GLA substrate resulted in an average conversion of 27% GLA to AA. Therefore, the GLA elongase has the ability to work with other enzymes in the n-6 PUFA synthetic pathway to produce desirable fatty acids.

To determine whether the above conversion is also true in n-3 pathways, the similar co-expression experiments were carried out in the presence of 25  $\mu$ M STA. Again, if both enzymes are expressed, the STA substrate will be converted to 20:4n-3 which will then be converted to EPA (20:5n-3) by the  $\Delta 5$ -desaturase. Figure 26B shows the results in which the production of EPA (approx. 40%) is observed. Once again, the GLA elongase demonstrates its ability to work with  $\Delta 5$ -desaturase in the n-3 pathway to produce desirable fatty acids.

### Example XII

#### Sequence Comparison Between GLELO and Other Fungal Elongases

The sequence analysis package of GCG (see Example I) was used to compare the GLELO sequence with known protein sequences. The nucleotide sequence of GLELO open reading frame was first translated into amino acid sequence that was used as a query sequence to search Swissprot database (see Example I) using the FastA algorithm (see Example I). Based on amino acid sequence similarity, the best matches were found with S. cerevisiae YJT6

(an EST with unknown annotation) with 33.9% identity in 189 amino acid overlap, S. cerevisiae ELO2 (GNS1) with 25.8% identity in 295 amino acid overlap, and S. cerevisiae ELO3 (SUR4) with 25.2% identity in 313 amino acid overlap. The FastA alignment of GLELO with MAELO showed 30.9% identity in 275 amino acids (Figure 27). GCG Pileup program creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments (see Example I), and was used with the elongases described above. The Pileup results indicate that there are many conserved regions among the elongases including a putative histidine box, which is underlined (Knutzon et. al., J. Biol. Chem. 273: 29360-29366, 1998) (Figure 28). Thus, although GLELO has similarity with MAELO, the difference in their encoded elongases may presumably be due to their substrate preference. GLA elongase can convert a higher percentage of GLA to DGLA than M. alpina elongase. In addition, MAELO expression in S. cerevisiae showed elongation of saturated and monounsaturated fatty acids in addition to GLA elongation to DGLA (see Example III).

#### Example XIII

##### Identification of M. alpina MAELO Homologues in Mammals

The MAELO translated sequence was used to search the Unified Human Transcript Database of Abbott Laboratories, 100 Abbott Park Rd., Abbott Park, Illinois 60064. This database was searched using Basic Local Alignment Search Tool (BLAST) (Altschul et al., Nuc. Acids Res. 25:3389-3402 (1997)) which "is a set of similarity search programs designed to explore all of the available sequence databases regardless of whether the query is a protein or DNA." Specifically, the tblastn algorithm was

used (i.e., a protein query search to a nucleotide database translated in six reading frames). The contig (CC) sequences in the Unified Human Transcript Database are consensus sequences representing groups of expressed sequence tags (EST) cDNAs  
5 derived from the public domain and from the Incyte LIFESEQ™ database of ESTs (Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304) that are clustered together on the basis of defined sequence homology, and assembled on the basis of sequence overlap. Two sequences from this database,  
10 CC067284R1 and CC1484548T1 had 28% identity in 242 amino acid overlap and 28.6% identity in 266 amino acid overlap, respectively, with the translated MAELO sequence. The two derived and edited sequences were designated as hs1 and hs2, respectively, and copied into the sequence analysis software  
15 package of GCG (see Example I). The translated MAELO sequence was aligned with translated HS1 (28.5% identity in 242 amino acids) and HS2 (28.2% identity in 266 amino acids) cDNA sequences using the FastA algorithm, as shown in Figures 29 and 30, respectively. HS1 cDNA nucleotide sequence also had 86.9%  
20 identity in 844 bp with the I05465 nucleotide sequence (see Example V). The translated HS2 cDNA sequence had 100% identity with the amino acid sequence from GenBank with accession number W74824 (see published PCT application WO9839448).

The National Center for Biotechnology Information (NCBI at  
25 <http://www.ncbi.nlm.nih.gov/>) was used to conduct database searches using tblastn with the 28 amino acid sequence (DTIFIILRKQKLIFLHWYHHITVLLYSW) translated from AC004050 (a human sequence identified in a TFastA search, see Example V). This amino acid sequence contains a histidine box (underlined), which  
30 has a noted motif of desaturases (Knutzon et al., supra), and both PUFA elongases, MAELO and GLELO (see Figure 28). A translated mouse sequence shown previously in Example V (GenBank

Accession #U97107) and a translated *C. elegans* sequence (GenBank Accession # U41011) had the highest matches with this 28 amino acid query. The NCBI mouse EST database was searched again with tblastn, using translated U41011 as a query. An additional  
5 mouse sequence was identified (GenBank Accession #AF014033.1), annotated as "putative involvement in fatty acid elongation." Three longer sequences (GenBank Accession #'s AA591034, AA189549, and AA839346) were identified through a tblastn search of the mouse EST database with translated AF014033.1 and  
10 combined into one sequence designated as mm2. The FastA alignment (see Example I) of translated mm2 and MAELO is shown in Figure 31. Another related, but not identical mouse sequence (GenBank Accession #AI225632), was also identified in a tblastn search of the mouse EST database with AF014033.1. The FastA  
15 alignment with translated AI225632 to MAELO is shown in Figure 32. The percent identity for both translated MM2 and AI 225632 with translated MAELO is 30.4% in 191 and 115 amino acid overlap, respectively. The level of amino acid identity with translated MAELO with these two translated mouse sequences  
20 identifies them as putative homologues of PUFA elongases.

#### Example XIV

##### Identification of *M. alpina* GLELO Homologues in Mammals

25

The TFastA algorithm, which compares a protein sequence to the database DNA sequence translated in each of the six reading frames, was used with translated GLELO as the query. The GenEMBL database from GCG was used to identify other potential  
30 elongase sequences based on their amino acid similarity to translated GLELO. Three human sequences were found to have matches with the GLELO amino acid sequence. These sequences



have GenBank accession numbers 1) AI815960, 2) AL034374, and 3) AC004050. AI815960, a Homo sapien EST sequence, has 40.3% identity in 144 amino acid overlap with translated GLELO (see Figure 33). A translated region of the human genomic sequence  
5 AL034374, derived from chromosome VI has 46.7% identity in a 60 amino acid overlap with translated GLELO. This homologous region in AL034374 appeared to be a part of the HS1 amino acid sequence which was shown to have homology with translated MAELO (see Example XIII). Therefore, HS1 sequence has similarity with  
10 both MAELO (see Figure 29) as well as GLELO (see Figure 34). A translated region of a human genomic sequence AC004050 from chromosome IV has 34.8% identity in 89 amino acid overlap with translated GLELO (see Figure 35). The amino acid identities between GLELO and these human sequences indicate that the  
15 proteins dervied from these human sequences could have related function, such as PUFA elongase activity.

To identify a mouse cDNA similar to GLELO, TFastA searches were performed with the GenEMBL database using translated GLELO as a query. From the TFastA searches, the three mouse sequences  
20 with the highest matches to translated GLELO were identified: (GenBank accession numbers 1) AF104033, 2) AI595258, and 3) U97107). AF104033 is annotated as "MUEL protein having putative fatty acid elongase with homology to yeast ELO3 (SUR4)" and is a part of the sequence of MM2. The MM2 sequence was initially  
25 derived from AF104033 mouse sequence, but the entire MM2 sequence was finally obtained through further mouse EST database searches and also shown to have homology with translated MAELO (see Example XIII and Figure 31).. When this MM2 amino acid sequence was aligned with translated GLELO sequence using FastA,  
30 a 34.6% identity in 211 amino acid overlap was found (see Figure 36) indicating that MM2 also has homology with GLELO. AI595258 is a mouse cDNA clone having 5' similarity with yeast ELO3

elongase and is part of mouse EST cDNA AI225632. The AI225632 mouse sequence, which is a longer sequence than AI595258, was shown to have similarity with translated MAELO (see Figure 32). The AI225632 was also aligned with the translated GLELO, and the FastA alignment is shown in Figure 37. A 35.3% identity in 199 amino acid overlap has been found. The third sequence, U97107, a mouse sequence, was annotated as "similar to yeast ELO3 (SUR4) gene." The FastA alignment of translated GLELO with U97107 is shown in Figure 38 where a 23.7% identity in 279 amino acid overlap was found. Previously, a region of U97107 was also found to have a high degree of homology with MAELO based on a FastA alignment (see Example V and Figure 16).

The above searches clearly indicate that the same human and mouse sequences were obtained by using either MAELO or GLELO as a query.

#### Example XV

#### Identification of M. alpina GLELO and MAELO Homologues in Other PUFA Producing Organisms

##### A) Caenorhabditis elegans:

A putative amino acid sequence deduced from a chromosomal sequence of C. elegans (GenBank Accession # U41011) was able to identify a partial sequence contained in the mouse MM2 putative PUFA elongase which has amino acid similarity with both GLA elongase (GLELO) and M. alpina elongase (MAELO). It was therefore conceivable that C. elegans homologues of GLELO or MAELO might be present in the nematode database. The putative amino acid sequences derived from GLELO and MAELO sequences were used as queries independently to search the nematode databases. A BLAST search (see Example XIII) was performed on wormpep16 (blastp compares an amino acid query sequence against a

nucleotide sequence database) and wormpep 16cDNAs (tblastn) databases which are predicted proteins and cDNAs obtained from the C. elegans genome sequencing project or EST's and their corresponding cDNA sequences, respectively. These sequence data were produced by the C. elegans Sequencing group, carried out jointly by the Sanger Centre and Genome Sequencing Center, and can be obtained from ftp://ftp.sanger.ac.uk/pub/databases/wormpep/. At least seven putative C. elegans translated sequences were identified by their amino acid sequence homology to the translated amino acid sequence of both GLELO and MAELO. The GenBank Accession #'s of those genomic sequences containing the deduced amino acids were identified as Z19154, U68749 (2 deduced proteins (F56H11.4 and F56H11.3 (wormpep Accession #'s)), U41011, U61954 (2 deduced proteins (F41H10.7 and F41H10.8 (wormpep Accession #'s)), and Z81058. Those underlined were identified in a previous search using translated MAELO as query (see Example V). As an example, the FastA amino acid alignments of translated U68749 (F56H11.4) with translated GLELO and MAELO are shown in Figures 39 and 40. Translated U68749 (F56H11.4) has 25-30% identity with both M. alpina elongase and GLA elongase in approximately a 200 amino acid overlap (see Figures 39 and 40). For all seven translated putative C. elegans cDNAs, the FastA alignments to translated GLELO was between 25-30% identity in a 200 amino acid overlap, while the identity was 26-34% in at least a 188 amino acid overlap for translated MAELO. The alignment similarities indicate that either translated GLELO or MAELO can be used to identify potential genes from C. elegans with elongase activity.

B) Drosophila melanogaster:

The translated deduced cDNA from the genomic sequence U41011 (C. elegans) had its highest match with a Drosophila

melanogaster EST, accession number AI134173 in a blastn search (compares a nucleotide query sequence against a nucleotide database) of the "other ESTs" database through NCBI (see Example XIII) and was assembled with an overlapping DNA EST fragment, accession number AI517255. The translated DNA fragment DM1, derived from the two overlapping sequences was aligned with translated GLELO as well as MAELO (see Figures 41 and 42) using FastA in GCG (see Example I). The alignments showed 27.2% identity with GLA elongase in a 206 amino acid overlap and 30% identity with M. alpina elongase in a 237 amino acid overlap. Thus, based on amino acid similarity, the DM1 could be a potential homologue to GLELO or MAELO having PUFA elongase-like activity. Moreover, using DNA sequences of GLELO and MAELO as queries for database searches, homologues with PUFA elongase activity from Drosophila can be identified.

#### Example XVI

##### Cloning and Expression of A Human PUFA Elongase Homologue

Many potential PUFA elongase sequences were identified based on their amino acid similarities to translated GLELO and/or MAELO. To determine the potential elongase activities of these sequences, the cDNA encoding the full length protein is then identified, cloned, and expressed, as demonstrated in the present example.

Primers R0719 (5' -GGT TCT CCC ATG GAA CAT TTT GAT GCA TC-3') and R0720 (5' -GGT TTC AAA GCT TTG ACT TCA ATC CTT CCG- 3') were designed based on the putative HS1 sequence, and used to amplify the human liver Marathon-Ready cDNA (Clontech Laboratories, Inc., Palo Alto, California). The polymerase Chain Reaction (PCR) was carried out in a 50  $\mu$ l volume containing: 5  $\mu$ l of human liver Marathon-Ready cDNA, 50 pmole each primer, 1 $\mu$ l 10 mM PCR Nucleotide Mix (Boehringer Mannheim

Corp., Indianapolis, IN), 5  $\mu$ l 10 X buffer and 1.0 U of Advantage KlenTaq Polymerase Mix (Clontech Laboratories, Inc., Palo Alto, CA). Thermocycler conditions in Perkin Elmer 9600 (Norwalk, CT) were as follows: 94 °C for 2 mins, then 30 cycles of 94 °C for 1 min., 58 °C for 2 mins, and 72 °C for 3 mins. PCR was followed by an additional extension cycle at 72 °C for 7 minutes.

The PCR amplified product was run on a gel, an amplified fragment of approximately 960 bp was gel purified, the termini of the fragment filled-in with T4 DNA polymerase (Boehringer Mannheim, Corp., Indianapolis, IN), and cloned into PCR-Blunt Vector (Invitrogen Corp., Carlsbad, CA) following manufacturer's protocol. The new plasmid was designated as pRAE-52, and the putative PUFA elongase cDNA in this clone was sequenced using ABI 373A Stretch DNA Sequencer (Perkin Elmer, Foster City, CA). The putative PUFA elongase cDNA sequence in plasmid pRAE-52 is shown in Figure 43, and the translated sequence is shown in Figure 44.

The putative PUFA elongase cDNA from plasmid pRAE-52 was then digested with *Nco*I/*Hind*III, gel purified, and ligated into pYX242(*Nco*I/*Hind*III). The new plasmid was designated as pRAE-58-A1. (Plasmid 58-A1 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on August 19, 1999, under the terms of the Budapest Treaty and was accorded deposit number \_\_\_\_\_.)

The construct pRAE-58-A1 was transformed into *S. cerevisiae* 334 (Hoveland et al., *supra*) and screened for elongase activity. The negative control strain was *S. cerevisiae* 334 containing pYX242 vector. The cultures were grown for 24 hours at 30°C, in selective media (Ausubel et al., *supra*), in the presence of 25  $\mu$ M of GLA or AA. In this study, DGLA or adrenic acid (ADA, 22:4n-6), respectively, was the predicted product of human elongase activity. When GLA was used as a substrate, the yeast cells

containing the human elongase cDNA contained elevated levels of DGLA compared to control cells, 2.75% vs. 0.09% of total fatty acids, respectively (see Figure 45). When AA was used as a substrate, the yeast cells containing the human elongase cDNA contained elevated levels of ADA compared to control cells, none detected vs. 1.21% of total fatty acids, respectively. Thus, the human elongase converts both 18 and 20 carbon chain long PUFAs to their respective elongated fatty acids.

The yeast cells containing the human elongase cDNA also had elevated levels of monounsaturated fatty acids including 18:1n-7, 20:1n-7, 20:1n-9, and 18:1n-5, compared to the control strain. Therefore, these results indicate that the identified human elongase is capable of utilizing PUFAs as well as monounsaturated fatty acids as substrates. Thus, this human sequence HSELO1, and its encoded protein, possess elongase activity independent of substrate specificity.

#### Example XVII

##### Cloning and Expression of a *C. elegans* PUFA Elongase

Several putative *C. elegans* elongases were identified with amino acid homology to both translated GLELO and MAELO. As with the human cDNA sequence, cloning of a cDNA and expression in yeast was used to determine if indeed it was a PUFA elongase. Primers RO738 (5' -AAT CAG GAA TTC ATG GCT CAG CAT CCG CTC GTT CAA C -3') and RO739 (5' -CCG CTT GTC GAC TTA GTT GTT CTT CTT CTT TGG CAC -3') with restriction sites *Eco*RI and *Sal*I (underlined), respectively, were based on the putative cDNA sequence contained in the genomic sequence U68749 (wormpep cDNA accession #F56H11.4.) A PCR amplification was performed in a 100  $\mu$ l volume containing: 250 ng excised *C. elegans* library cDNA (OriGene Technologies Inc., Rockville, MD), 50 pmole each

primer, 10  $\mu$ l 10X reaction buffer (Boehringer Mannheim Corp., Indianapolis, IN), 1  $\mu$ l 10 mM PCR Nucleotide mix (Boehringer Mannheim Corp., Indianapolis, IN), and 2.5 U Taq polymerase (Boehringer Mannheim Corp., Indianapolis, IN). Thermocycler conditions in a Perkin Elmer 9600 (Norwalk, CT) were as follows: 95 °C for 5 mins, then 25 cycles of 94 °C for 30 secs, 55 °C for 2 mins, and 72 °C for 2 mins. PCR was followed by an additional cycle of 72 °C for 7 minutes.

The PCR amplified product was purified from an agarose gel, cut with *EcoRI* and *SalI*, ligated to pYX242 (Invitrogen Corp., Carlsbad, CA) (linearized with *EcoRI* and *SalI*) using the Rapid Ligation kit (Boehringer Mannheim Corp., Indianapolis, IN), according to the manufacturer's protocol and transformed into *E. coli* Top10 cells (Invitrogen Corp., Carlsbad, CA). The new plasmids, designated pRET-21 and pRET-22 (two individual clones from the ligation), were sequenced with the 373A Stretch DNA sequencer ABI (Perkin Elmer, Foster City, CA), and the cDNA sequences were identical. The 867 base cDNA nucleotide sequence of the plasmid pRET-22 containing the putative elongase is shown in Figure 46 and the translated sequence of 288 amino acids is shown in Figure 47. (Plasmid pRET-22 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on August 19, 1999, under the terms of the Budapest Treaty and was accorded deposit number \_\_\_\_.)

The plasmids pRET-21 and -22 were transformed into *S. cerevisiae* 334 as previously described (see Example III) and the resulting yeast cultures (334(pRET-21) and 334(pRET-22)) grown in 100 ml of selective media without leucine (Ausubel et al, *supra*) for 48 hours at 20 °C in the presence of 50  $\mu$ M GLA and AA. The cell pellets were collected and subjected to fatty acid analysis and the results shown in Figure 48. DGLA, the

predicted product from GLA elongation, was found to be an average of 1.79% of the total lipid in the two samples, versus 0.13% for the negative control (334 containing plasmid pYX242) indicating that the enzyme encoded by both pRET-21 and pRET-22 possessed GLA elongase activity. The percent conversion of GLA to DGLA by 334(pRET-21) and 334(pRET-22) was 11.1% and 19.4% respectively with an average of 15.25%. Interestingly, almost no elongation of AA or any endogenous fatty acid was observed (Fig. 48). These results indicate that the elongase encoded by this newly identified C. elegans cDNA, CEEL01, is able to specifically elongate GLA to DGLA, suggesting that it may be a C. elegans homologue of GLA elongase.

#### Example VIII

##### Isolation of a Putative Human Elongase cDNA Based on AC004050 Sequence

To isolate the full length putative elongase cDNA based on the AC004050 sequence, primers RP735 (5' -CCT CCT GAA TTC CAQA CAC TAT TCA GCT TTC -3') and R073 (5' -TAA TAC GAC TCA CTA TAG GG -3') were used to PCR amplify the human liver Marathon-Ready cDNA (Clontech Laboratories, Inc., Palo Alto, CA). The PCR was carried out using the Advantage<sup>TM</sup> cDNA PCR Kit (Clontech Laboratories, Inc., Palo Alto, CA) with 5  $\mu$ l of human liver Marathon-Ready cDNA and 50 pmole each primer following manufacturer's instructions. Thermocycler conditions in Perkin Elmer 9600 (Norwalk, CT) were as follows: 94 °C for 2 mins, then 30 cycles of 94 °C for 1 min., 58 °C for 2 mins., and 72 °C for 3 mins. PCR was followed by an additional extension at 72 °C for 7 mins.

The PCR amplified product was run on a gel, an amplified fragment of approximately 1 Kb was gel purified, the termini of



the fragment were filled in with T4DNA polymerase (Boehringer Mannheim, Corp., Carlsbad, CA) following manufacturer's instructions. The new plasmid was designated as pRAE-59, and the putative PUFA elongase cDNA in this plasmid, designated as HS3, was sequenced using the ABI 373A Stretch Sequencer (Perkin Elmer, Foster City, CA). The putative PUFA elongase cDNA sequence HS3 is shown in Figure 49, and the translated sequence is shown in Figure 50.

#### Nutritional Compositions

The PUFAs described in the Detailed Description may be utilized in various nutritional supplements, infant formulations, nutritional substitutes and other nutritional solutions.

#### I. INFANT FORMULATIONS

##### A. Isomil® Soy Formula with Iron:

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cows milk. A feeding for patients with disorders for which lactose should be avoided: lactase deficiency, lactose intolerance and galactosemia.

##### Features:

- Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.
- Lactose-free formulation to avoid lactose-associated diarrhea.
- Low osmolality (240 mOs/kg water) to reduce risk of osmotic diarrhea.
- Dual carbohydrates (corn syrup and sucrose) designed to

enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.

-1.8 mg of Iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.

5 -Recommended levels of vitamins and minerals.

-Vegetable oils to provide recommended levels of essential fatty acids.

-Milk-white color, milk-like consistency and pleasant aroma.

10

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6% sugar (sucrose), 2.1 % soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0.11 % calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy  
15 lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m- inositol, alpha-tocopheryl acetate, zinc sulfate, L-  
20 carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

25

B. Isomil® DF Soy Formula For Diarrhea:

Usage: As a short-term feeding for the dietary management of diarrhea in infants and toddlers.

30

Features:

-First infant formula to contain added dietary fiber from soy fiber specifically for diarrhea management.

-Clinically shown to reduce the duration of loose, watery stools during mild to severe diarrhea in infants.

5 -Nutritionally complete to meet the nutritional needs of the infant.

-Soy protein isolate with added L-methionine meets or exceeds an infant's requirement for all essential amino acids.

10 -Lactose-free formulation to avoid lactose-associated diarrhea.

-Low osmolality (240 mOsm/kg water) to reduce the risk of osmotic diarrhea.

15 -Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.

-Meets or exceeds the vitamin and mineral levels recommended by the Committee on Nutrition of the American Academy of Pediatrics and required by the Infant Formula Act.

20 -1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.

-Vegetable oils to provide recommended levels of essential fatty acids.

25

Ingredients: (Pareve) 86% water, 4.8% com syrup, 2.5% sugar (sucrose), 2.1% soy oil, 2.0% soy protein isolate, 1.4% coconut oil, 0.77% soy fiber, 0.12% calcium citrate, 0.11 % calcium phosphate tribasic, 0.10% potassium citrate, 30 potassium chloride, potassium phosphate monobasic, mono and diglycerides, soy lecithin, carrageenan, magnesium chloride, ascorbic acid, L-methionine, potassium phosphate

dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride  
5 hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

C. Isomil® SF Sucrose-Free Soy Formula With Iron:

10

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cow's-milk protein or an intolerance to sucrose. A feeding for patients with disorders for which lactose and sucrose should be avoided.

15

Features:

- Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.
- Lactose-free formulation to avoid lactose-associated  
20 diarrhea (carbohydrate source is Polycose® Glucose Polymers).
- Sucrose free for the patient who cannot tolerate sucrose.
- Low osmolality (180 mOsm/kg water) to reduce risk of osmotic diarrhea.
- 25 -1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- Recommended levels of vitamins and minerals.
- Vegetable oils to provide recommended levels of essential fatty acids.
- 30 -Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve) 75% water, 11.8% hydrolyzed  
cornstarch, 4.1% soy oil, 4.1 % soy protein isolate, 2.8%  
coconut oil, 1.0% modified cornstarch, 0.38% calcium  
phosphate tribasic, 0. 17% potassium citrate, 0.13%  
5 potassium chloride, mono- and diglycerides, soy lecithin,  
magnesium chloride, ascorbic acid, L-methionine, calcium  
carbonate, sodium chloride, choline chloride,  
carrageenan, taurine, ferrous sulfate, m-inositol, alpha-  
tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide,  
10 calcium pantothenate, cupric sulfate, vitamin A palmitate,  
thiamine chloride hydrochloride, riboflavin, pyridoxine  
hydrochloride, folic acid, manganese sulfate, potassium  
iodide, phylloquinone, biotin, sodium selenite, vitamin D3  
and cyanocobalamin.

15

D. Isomil® 20 Soy Formula With Iron Ready To Feed,  
20 Cal/fl oz.:

Usage: When a soy feeding is desired.

20 Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6%  
sugar(sucrose), 2.1 % soy oil, 1.9% soy protein isolate,  
1.4% coconut oil, 0.15% calcium citrate, 0. 11% calcium  
phosphate tribasic, potassium citrate, potassium phosphate  
monobasic, potassium chloride, mono- and diglycerides, soy  
25 lecithin, carrageenan, ascorbic acid, L-methionine,  
magnesium chloride, potassium phosphate dibasic, sodium  
chloride, choline chloride, taurine, ferrous sulfate, m-  
inositol, alpha-tocopheryl acetate, zinc sulfate, L-  
carnitine, niacinamide, calcium pantothenate, cupric  
30 sulfate, vitamin A palmitate, thiamine chloride  
hydrochloride, riboflavin, pyridoxine hydrochloride, folic

acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

E. Similac® Infant Formula:

5

Usage: When an infant formula is needed: if the decision is made to discontinue breastfeeding before age 1 year, if a supplement to breastfeeding is needed or as a routine feeding if breastfeeding is not adopted.

10

Features:

-Protein of appropriate quality and quantity for good growth; heat-denatured, which reduces the risk of milk-associated enteric blood loss.

15 -Fat from a blend of vegetable oils (doubly homogenized), providing essential linoleic acid that is easily absorbed.

-Carbohydrate as lactose in proportion similar to that of human milk.

20 -Low renal solute load to minimize stress on developing organs.

-Powder, Concentrated Liquid and Ready To Feed forms.

Ingredients: (-D) Water, nonfat milk, lactose, soy oil, coconut oil, mono- and diglycerides, soy lecithin,  
25 ascorbic acid, carrageenan, choline chloride, taurine, m-  
inositol, alpha-tocopheryl acetate, zinc sulfate,  
niacinamide, ferrous sulfate, calcium pantothenate, cupric  
sulfate, vitamin A palmitate, thiamine chloride  
hydrochloride, riboflavin, pyridoxine hydrochloride, folic  
30 acid, manganese sulfate, phylloquinone, biotin, sodium  
selenite, vitamin D3 and cyanocobalamin.

F. Similac® NeoCare Premature Infant Formula With Iron:

Usage: For premature infants' special nutritional needs after hospital discharge. Similac NeoCare is a  
5 nutritionally complete formula developed to provide premature infants with extra calories, protein, vitamins and minerals needed to promote catch-up growth and support development.

10 Features:

-Reduces the need for caloric and vitamin supplementation. More calories (22 Cal/fl oz) than standard term formulas (20 Cal/fl oz).

-Highly absorbed fat blend, with medium-chain triglycerides  
15 (MCT oil) to help meet the special digestive needs of premature infants.

-Higher levels of protein, vitamins and minerals per 100 calories to extend the nutritional support initiated in-hospital.

20 -More calcium and phosphorus for improved bone mineralization.

Ingredients: -D Corn syrup solids, nonfat milk, lactose, whey protein concentrate, soy oil, high-oleic safflower  
25 oil, fractionated coconut oil (medium chain triglycerides), coconut oil, potassium citrate, calcium phosphate tribasic, calcium carbonate, ascorbic acid, magnesium chloride, potassium chloride, sodium chloride, taurine, ferrous sulfate, m-inositol, choline chloride, ascorbyl  
30 palmitate, L-carnitine, alpha-tocopheryl acetate, zinc sulfate, niacinamide, mixed tocopherols, sodium citrate, calcium pantothenate, cupric sulfate, thiamine chloride

hydrochloride, vitamin A palmitate, beta carotene, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

5

G. Similac Natural Care Low-Iron Human Milk Fortifier Ready To Use, 24 Cal/fl oz.:

Usage: Designed to be mixed with human milk or to be fed  
10 alternatively with human milk to low-birth-weight infants.

15

Ingredients: -D Water, nonfat milk, hydrolyzed cornstarch, lactose, fractionated coconut oil (medium-chain triglycerides), whey protein concentrate, soy oil, coconut  
oil, calcium phosphate tribasic, potassium citrate,  
magnesium chloride, sodium citrate, ascorbic acid, calcium  
carbonate, mono and diglycerides, soy lecithin,  
carrageenan, choline chloride, m-inositol, taurine,  
niacinamide, L-carnitine, alpha tocopheryl acetate, zinc  
20 sulfate, potassium chloride, calcium pantothenate, ferrous  
sulfate, cupric sulfate, riboflavin, vitamin A palmitate,  
thiamine chloride hydrochloride, pyridoxine hydrochloride,  
biotin, folic acid, manganese sulfate, phylloquinone,  
vitamin D3, sodium selenite and cyanocobalamin.

25

Various PUFAs of this invention can be substituted and/or added to the infant formulae described above and to other infant formulae known to those in the art.

## 30 II. NUTRITIONAL FORMULATIONS

### A. ENSURE®



Usage: ENSURE is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE is lactose- and gluten-free, and is  
5 suitable for use in modified diets, including low-cholesterol diets. Although it is primarily an oral supplement, it can be fed by tube.

Patient Conditions:

- 10 -For patients on modified diets
- For elderly patients at nutrition risk
- For patients with involuntary weight loss
- For patients recovering from illness or surgery
- For patients who need a low-residue diet

15  
Ingredients: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate,  
20 Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate,  
25 Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate.

30 B. ENSURE® BARS:

Usage: ENSURE BARS are complete, balanced nutrition for

supplemental use between or with meals. They provide a delicious, nutrient-rich alternative to other snacks. ENSURE BARS contain <1 g lactose/bar, and Chocolate Fudge Brownie flavor is gluten-free. (Honey Graham Crunch flavor  
5 contains gluten.)

Patient Conditions:

- For patients who need extra calories, protein, vitamins and minerals.
- 10 -Especially useful for people who do not take in enough calories and nutrients.
- For people who have the ability to chew and swallow
- Not to be used by anyone with a peanut allergy or any type of allergy to nuts.

- 15 Ingredients: Honey Graham Crunch -- High-Fructose Corn Syrup, Soy Protein Isolate, Brown Sugar, Honey, Maltodextrin (Corn), Crisp Rice (Milled Rice, Sugar [Sucrose], Salt [Sodium Chloride] and Malt), Oat Bran, Partially Hydrogenated Cottonseed and Soy Oils, Soy  
20 Polysaccharide, Glycerine, Whey Protein Concentrate, Polydextrose, Fructose, Calcium Caseinate, Cocoa Powder, Artificial Flavors, Canola Oil, High-Oleic Safflower Oil, Nonfat Dry Milk, Whey Powder, Soy Lecithin and Corn Oil. Manufactured in a facility that processes  
25 nuts.

- Vitamins and Minerals: Calcium Phosphate Tribasic, Potassium Phosphate Dibasic, Magnesium Oxide, Salt (Sodium Chloride), Potassium Chloride, Ascorbic Acid, Ferric  
30 Orthophosphate, Alpha-Tocopheryl Acetate, Niacinamide, Zinc Oxide, Calcium Pantothenate, Copper Gluconate, Manganese

Sulfate, Riboflavin, Beta Carotene, Pyridoxine  
Hydrochloride, Thiamine Mononitrate, Folic Acid, Biotin,  
Chromium Chloride, Potassium Iodide, Sodium Selenate,  
Sodium Molybdate, Phylloquinone, Vitamin D3 and  
5 Cyanocobalamin.

Protein: Honey Graham Crunch - The protein source is a  
blend of soy protein isolate and milk proteins.

10

Soy protein isolate	74%
Milk proteins	26%

Fat: Honey Graham Crunch - The fat source is a blend of  
15 partially hydrogenated cottonseed and soybean, canola, high  
oleic safflower, oils, and soy lecithin.

Partially hydrogenated cottonseed and soybean oil	76%
Canola oil	8%
20 High-oleic safflower oil	8%
Corn oil	4%
Soy lecithin	4%

Carbohydrate: Honey Graham Crunch - The carbohydrate source  
25 is a combination of high-fructose corn syrup, brown sugar,  
maltodextrin, honey, crisp rice, glycerine, soy  
polysaccharide, and oat bran.

High-fructose corn syrup	24%
30 Brown sugar	21%
Maltodextrin	12%
Honey	11%

Crisp rice	9%
Glycerine	9%
Soy Polysaccharide	7%
Oat bran	7%

5

## C. ENSURE® HIGH PROTEIN:

Usage: ENSURE HIGH PROTEIN is a concentrated, high-protein liquid food designed for people who require additional calories, protein, vitamins, and minerals in their diets. It can be used as an oral nutritional supplement with or between meals or, in appropriate amounts, as a meal replacement. ENSURE HIGH PROTEIN is lactose- and gluten-free, and is suitable for use by people recovering from general surgery or hip fractures and by patients at risk for pressure ulcers.

Patient Conditions:

- For patients who require additional calories, protein, vitamins, and minerals, such as patients recovering from general surgery or hip fractures, patients at risk for pressure ulcers, and patients on low-cholesterol diets

Features:

- Low in saturated fat
- Contains 6 g of total fat and < 5 mg of cholesterol per serving
- Rich, creamy taste
- Excellent source of protein, calcium, and other essential vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

## Ingredients:

Vanilla Supreme: -D Water, Sugar (Sucrose), Maltodextrin  
5 (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower  
Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium  
Citrate, Calcium Phosphate Tribasic, Sodium Citrate,  
Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial  
Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride,  
10 Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate,  
Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide,  
Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate,  
Vitamin A Palmitate, Thiamine Chloride Hydrochloride,  
Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium  
15 Molybdate, Chromium Chloride, Biotin, Potassium Iodide,  
Sodium Selenate, Phylloquinone, Vitamin D3 and  
Cyanocobalamin.

## Protein:

20 The protein source is a blend of two high-biologic-  
value proteins: casein and soy.

Sodium and calcium caseinates 85%  
Soy protein isolate 15%

25

## Fat:

The fat source is a blend of three oils: high-oleic  
safflower, canola, and soy.

30 High-oleic safflower oil 40%  
Canola oil 30%  
Soy oil 30%

The level of fat in ENSURE HIGH PROTEIN meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE HIGH PROTEIN represent 24% of the total calories, with 2.6% of the fat being from saturated fatty acids and 7.9% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

#### Carbohydrate:

ENSURE HIGH PROTEIN contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla supreme, chocolate royal, wild berry, and banana), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

#### 20 Vanilla and other nonchocolate flavors:

Sucrose	60%
Maltodextrin	40%

#### 25 Chocolate:

Sucrose	70%
Maltodextrin	30%

30

#### D. ENSURE® LIGHT

Usage: ENSURE LIGHT is a low-fat liquid food designed for use as an oral nutritional supplement with or between meals. ENSURE LIGHT is lactose- and gluten-free, and is suitable for use in modified diets, including low-  
5 cholesterol diets.

Patient Conditions:

-For normal-weight or overweight patients who need extra  
10 nutrition in a supplement that contains 50% less fat and 20% fewer calories than ENSURE.

-For healthy adults who don't eat right and need extra nutrition.

Features:

15

-Low in fat and saturated fat

-Contains 3 g of total fat per serving and < 5 mg cholesterol

-Rich, creamy taste

20 -Excellent source of calcium and other essential vitamins and minerals

-For low-cholesterol diets

-Lactose-free, easily digested

25 Ingredients:

French Vanilla: -D Water, Maltodextrin (Corn), Sugar (Sucrose), Calcium Caseinate, High-Oleic Safflower Oil, Canola Oil, Magnesium Chloride, Sodium Citrate, Potassium  
30 Citrate, Potassium Phosphate Dibasic, Magnesium Phosphate Dibasic, Natural and Artificial Flavor, Calcium Phosphate Tribasic, Cellulose Gel, Choline Chloride, Soy Lecithin,

Carrageenan, Salt (Sodium Chloride), Ascorbic Acid,  
Cellulose Gum, Ferrous Sulfate, Alpha-Tocopheryl Acetate,  
Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium  
Pantothenate, Cupric Sulfate, Thiamine Chloride  
5 Hydrochloride, Vitamin A Palmitate, Pyridoxine  
Hydrochloride, Riboflavin, Chromium Chloride, Folic Acid,  
Sodium Molybdate, Biotin, Potassium Iodide, Sodium  
Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

10 Protein:

The protein source is calcium caseinate.

Calcium caseinate	100%
-------------------	------

15 Fat:

The fat source is a blend of two oils: high-oleic safflower  
and canola.

20 High-oleic safflower oil	70%
Canola oil	30%

The level of fat in ENSURE LIGHT meets American Heart  
Association (AHA) guidelines. The 3 grams of fat in ENSURE  
25 LIGHT represent 13.5% of the total calories, with 1.4% of  
the fat being from saturated fatty acids and 2.6%  
from polyunsaturated fatty acids. These values are within  
the AHA guidelines of < 30% of total calories from fat, <  
10% of the, calories from saturated fatty acids, and < 10%  
30 of total calories from polyunsaturated fatty acids.

Carbohydrate:



ENSURE LIGHT contains a combination of maltodextrin and sucrose. The chocolate flavor contains corn syrup as well. The mild sweetness and flavor variety (French vanilla, chocolate supreme, strawberry swirl), plus VARI-FLAVORS®  
5 Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors:

10

Sucrose	51%
Maltodextrin	49%

Chocolate:

15

Sucrose	47.0%
Corn Syrup	26.5%
Maltodextrin	26.5%

20 Vitamins and Minerals:

An 8-fl-oz serving of ENSURE LIGHT provides at least 25% of the RDIs for 24 key vitamins and minerals.

Caffeine:

25 Chocolate flavor contains 2.1 mg caffeine/8 fl oz.

E. ENSURE PLUS®

Usage: ENSURE PLUS is a high-calorie, low-residue liquid food for use when extra calories and nutrients, but a  
30 normal concentration of protein, are needed. It is designed primarily as an oral nutritional supplement to be used

with or between meals or, in appropriate amounts, as a meal replacement. ENSURE PLUS is lactose- and gluten-free. Although it is primarily an oral nutritional supplement, it can be fed by tube.

5

Patient Conditions:

- For patients who require extra calories and nutrients, but a normal concentration of protein, in a limited volume
- For patients who need to gain or maintain healthy weight

10

Features:

- Rich, creamy taste
- Good source of essential vitamins and minerals

15 

Ingredients:

Vanilla: -D Water, Corn Syrup, Maltodextrin (Corn), Corn Oil, Sodium and Calcium Caseinates, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Potassium Chloride, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D3.

30

Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

	Sodium and calcium caseinates	84%
5	Soy protein isolate	16%

Fat:

The fat source is corn oil.

10	Corn oil	100%
----	----------	------

Carbohydrate:

ENSURE PLUS contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, strawberry, coffee, butter pecan, and eggnog), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

20 Vanilla, strawberry, butter pecan, and coffee flavors:

	Corn Syrup	39%
	Maltodextrin	38%
	Sucrose	23%

25

Chocolate and eggnog flavors:

	Corn Syrup	36%
	Maltodextrin	34%
30	Sucrose	30%

#### Vitamins and Minerals:

An 8-fl-oz serving of ENSURE PLUS provides at least 15% of the RDIs for 25 key Vitamins and minerals.

#### 5 Caffeine:

Chocolate flavor contains 3.1 mg Caffeine/8 fl oz.  
Coffee flavor contains a trace amount of caffeine.

#### F. ENSURE PLUS® HN

10

Usage: ENSURE PLUS HN is a nutritionally complete high-calorie, high-nitrogen liquid food designed for people with higher calorie and protein needs or limited volume tolerance. It may be used for oral supplementation or for  
15 total nutritional support by tube. ENSURE PLUS HN is lactose- and gluten-free.

#### Patient Conditions:

- For patients with increased calorie and protein needs,  
20 such as following surgery or injury.
- For patients with limited volume tolerance and early satiety.

#### Features:

- 25 -For supplemental or total nutrition
- For oral or tube feeding
- 1.5 CaVmL,
- High nitrogen
- Calorically dense

30

#### Ingredients:

Vanilla: -D Water, Maltodextrin (Corn), Sodium and Calcium Caseinates, Corn Oil, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor,  
5 Sodium Citrate, Choline Chloride, Ascorbic Acid, Taurine, L-Carnitine, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Carrageenan, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride,  
10 Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D3.

15 G. ENSURE® POWDER:

Usage: ENSURE POWDER (reconstituted with water) is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals. ENSURE POWDER  
20 is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:

- For patients on modified diets
- 25 -For elderly patients at nutrition risk
- For patients recovering from illness/surgery
- For patients who need a low-residue diet

Features:

- 30 -Convenient, easy to mix
- Low in saturated fat

- Contains 9 g of total fat and < 5 mg of cholesterol per serving
- High in vitamins and minerals
- For low-cholesterol diets
- 5 -Lactose-free, easily digested

Ingredients: -D Corn Syrup, Maltodextrin (Corn), Sugar (Sucrose), Corn Oil, Sodium and Calcium Caseinates, Soy Protein Isolate, Artificial Flavor, Potassium Citrate, 10 Magnesium Chloride, Sodium Citrate, Calcium Phosphate Tribasic, Potassium Chloride, Soy Lecithin, Ascorbic Acid, Choline Chloride, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Thiamine Chloride Hydrochloride, Cupric 15 Sulfate, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Sodium Molybdate, Chromium Chloride, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

20 Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

	Sodium and calcium caseinates	84%
25	Soy protein isolate	16%

Fat:

The fat source is corn oil.

30	Corn oil	100%
----	----------	------

**Carbohydrate:**

ENSURE POWDER contains a combination of corn syrup, maltodextrin, and sucrose. The mild sweetness of ENSURE POWDER, plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, 5 strawberry, lemon, and orange, helps to prevent flavor fatigue and aid in patient compliance.

**Vanilla:**

10	Corn Syrup	35%
	Maltodextrin	35%
	Sucrose	30%

**H. ENSURE® PUDDING**

15

Usage: ENSURE PUDDING is a nutrient-dense supplement providing balanced nutrition in a nonliquid form to be used with or between meals. It is appropriate for consistency-modified diets (e.g., soft, pureed, or full liquid) or for 20 people with swallowing impairments. ENSURE PUDDING is gluten-free.

**Patient Conditions:**

- For patients on consistency-modified diets (e.g., soft, 25 pureed, or full liquid)
- For patients with swallowing impairments

**Features:**

- Rich and creamy, good taste
- 30 -Good source of essential vitamins and minerals
- Convenient-needs no refrigeration
- Gluten-free

Nutrient Profile per 5 oz: Calories 250, Protein 10.9%,  
Total Fat 34.9%, Carbohydrate 54.2%

5   Ingredients:

Vanilla: -D Nonfat Milk, Water, Sugar (Sucrose), Partially  
Hydrogenated Soybean Oil, Modified Food Starch, Magnesium  
Sulfate, Sodium Stearoyl Lactylate, Sodium Phosphate  
Dibasic, Artificial Flavor, Ascorbic Acid, Zinc Sulfate,  
10   Ferrous Sulfate, Alpha-Tocopheryl Acetate, Choline  
Chloride, Niacinamide, Manganese Sulfate, Calcium  
Pantothenate, FD&C Yellow #5, Potassium Citrate, Cupric  
Sulfate, Vitamin A Palmitate, Thiamine Chloride  
Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, FD&C  
15   Yellow #6, Folic Acid, Biotin, Phylloquinone, Vitamin D3  
and Cyanocobalamin.

Protein:

20       The protein source is nonfat milk.

Nonfat milk	100%
-------------	------

Fat:

25       The fat source is hydrogenated soybean oil.

Hydrogenated soybean oil	100%
--------------------------	------

Carbohydrate:

30

ENSURE PUDDING contains a combination of sucrose and  
modified food starch. The mild sweetness and flavor variety



(vanilla, chocolate, butterscotch, and tapioca) help prevent flavor fatigue. The product contains 9.2 grams of lactose per serving.

5 Vanilla and other nonchocolate flavors:

Sucrose	56%
Lactose	27%
Modified food starch	17%

10

Chocolate:

Sucrose	58%
Lactose	26%
15 Modified food starch	16%

I. ENSURE® WITH FIBER:

Usage: ENSURE WITH FIBER is a fiber-containing, nutritionally complete liquid food designed for people who  
20 can benefit from increased dietary fiber and nutrients.  
ENSURE WITH FIBER is suitable for people who do not require a low-residue diet. It can be fed orally or by tube, and can be used as a nutritional supplement to a regular diet or, in appropriate amounts, as a meal replacement. ENSURE  
25 WITH FIBER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:

-For patients who can benefit from increased dietary fiber  
30 and nutrients

Features:

- New advanced formula-low in saturated fat, higher in vitamins and minerals
- Contains 6 g of total fat and < 5 mg of cholesterol per serving
- 5 -Rich, creamy taste
- Good source of fiber
- Excellent source of essential vitamins and minerals
- For low-cholesterol diets
- Lactose- and gluten-free

10

#### Ingredients:

- Vanilla: -D Water; Maltodextrin (Corn), Sugar (Sucrose), Sodium and Calcium Caseinates, Oat Fiber, High-Oleic Safflower Oil, Canola Oil, Soy Protein Isolate, Corn Oil,
- 15 Soy Fiber, Calcium Phosphate Tribasic, Magnesium Chloride, Potassium Citrate, Cellulose Gel, Soy Lecithin, Potassium Phosphate Dibasic, Sodium Citrate, Natural and Artificial Flavors, Choline Chloride, Magnesium Phosphate, Ascorbic Acid, Cellulose Gum, Potassium Chloride, Carrageenan,
- 20 Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Chromium Chloride, Biotin, Sodium Molybdate,
- 25 Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

#### Protein:

- The protein source is a blend of two high-biologic-
- 30 value proteins-casein and soy.

Sodium and calcium caseinates

80%

Soy protein isolate 20%

Fat:

5 The fat source is a blend of three oils: high-oleic safflower, canola, and corn.

High-oleic safflower oil	40%
Canola oil	40%
10 Corn oil	20%

The level of fat in ENSURE WITH FIBER meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE WITH FIBER represent 22% of the total calories, with 2.01 %  
15 of the fat being from saturated fatty acids and 6.7% from polyunsaturated fatty acids. These values are within the AHA guidelines of  $\leq 30\%$  of total calories from fat,  $< 10\%$  of the calories from saturated fatty acids, and  $\leq 10\%$  of total calories from polyunsaturated fatty acids.

20

Carbohydrate:

ENSURE WITH FIBER contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, and butter pecan), plus VARI-  
25 FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors:

30

Maltodextrin	66%
Sucrose	25%

Oat Fiber	7%
Soy Fiber	2%

## Chocolate:

5

Maltodextrin	55%
Sucrose	36%
Oat Fiber	7%
Soy Fiber	2%

10

## Fiber:

The fiber blend used in ENSURE WITH FIBER consists of oat fiber and soy polysaccharide. This blend results in approximately 4 grams of total dietary fiber per 8-fl. oz can. The ratio of insoluble to soluble fiber is 95:5.

15

The various nutritional supplements described above and known to others of skill in the art can be substituted and/or supplemented with the PUFAs produced in accordance with the present invention.

20

## J. Oxepa™ Nutritional Product

Oxepa is a low-carbohydrate, calorically dense, enteral nutritional product designed for the dietary management of patients with or at risk for ARDS. It has a unique combination of ingredients, including a patented oil blend containing eicosapentaenoic acid (EPA from fish oil),  $\gamma$ -linolenic acid (GLA from borage oil), and elevated antioxidant levels.

30

## Caloric Distribution:

Caloric density is high at 1.5 Cal/mL (355 Cal/8 fl oz), to minimize the volume required to meet energy needs. The distribution of Calories in Oxepa is shown in Table IV.

5                    Table IV. Caloric Distribution of Oxepa

	per 8 fl oz.	per liter	% of Cal
Calories	355	1,500	---
Fat (g)	22.2	93.7	55.2
Carbohydrate (g)	25	105.5	28.1
10 Protein (g)	14.8	62.5	16.7
Water (g)	186	785	---

Fat:

-Oxepa contains 22.2 g of fat per 8-fl oz serving (93.7 g/L).

-The fat source is an oil blend of 31.8% canola oil, 25% medium-chain triglycerides (MCTs), 20% borage oil, 20% fish oil, and 3.2 % soy lecithin. The typical fatty acid profile of Oxepa is shown in Table V.

20 -Oxepa provides a balanced amount of polyunsaturated, monounsaturated, and saturated fatty acids, as shown in Table VI.

25 -Medium-chain triglycerides (MCTs) -- 25% of the fat blend -- aid gastric emptying because they are absorbed by the intestinal tract without emulsification by bile acids.

30 The various fatty acid components of Oxepa™ nutritional product can be substituted and/or supplemented with the PUFAs produced in accordance with this invention.

Table V. Typical Fatty Acid Profile

		% Total	g/8 fl oz*	g/L*
5	Fatty Acids			
	Caproic (6:0)	0.2	0.04	0.18
	Caprylic (8:0)	14.69	3.1	13.07
10	Capric (10:0)	11.06	2.33	9.87
	Palmitic (16:0)	5.59	1.18	4.98
	Palmitoleic	1.82	0.38	1.62
	Stearic	1.94	0.39	1.64
	Oleic	24.44	5.16	21.75
15	Linoleic	16.28	3.44	14.49
	$\alpha$ -Linolenic	3.47	0.73	3.09
	$\gamma$ -Linolenic	4.82	1.02	4.29
	Eicosapentaenoic	5.11	1.08	4.55
	n-3-Docosapent-	0.55	0.12	0.49
20	aenoic			
	Docosahexaenoic	2.27	0.48	2.02
	Others	7.55	1.52	6.72

25 Fatty acids equal approximately 95% of total fat.

Table VI. Fat Profile of Oxepa.

% of total calories from fat	55.2
Polyunsaturated fatty acids	31.44 g/L
Monounsaturated fatty acids	25.53 g/L
5 Saturated fatty acids	32.38 g/L
n-6 to n-3 ratio	1.75:1
Cholesterol	9.49 mg/8 fl oz
	40.1 mg/L

## Carbohydrate:

- 10 -The carbohydrate content is 25.0 g per 8-fl-oz serving (105.5 g/L).
- The carbohydrate sources are 45% maltodextrin (a complex carbohydrate) and 55% sucrose (a simple sugar), both of which are readily digested and absorbed.
- 15 -The high-fat and low-carbohydrate content of Oxepa is designed to minimize carbon dioxide (CO<sub>2</sub>) production. High CO<sub>2</sub> levels can complicate weaning in ventilator-dependent patients. The low level of carbohydrate also may be useful for those patients who have developed stress-induced
- 20 hyperglycemia.
- Oxepa is lactose-free.

Dietary carbohydrate, the amino acids from protein, and the glycerol moiety of fats can be converted to glucose

25 within the body. Throughout this process, the carbohydrate requirements of glucose-dependent tissues (such as the central nervous system and red blood cells) are met.

However, a diet free of carbohydrates can lead to ketosis, excessive catabolism of tissue protein, and loss of fluid

30 and electrolytes. These effects can be prevented by daily ingestion of 50 to 100 g of digestible carbohydrate, if caloric intake is adequate. The carbohydrate level in Oxepa

is also sufficient to minimize gluconeogenesis, if energy needs are being met.

Protein:

5 -Oxepa contains 14.8 g of protein per 8-fl-oz serving (62.5 g/L).

-The total calorie/nitrogen ratio (150:1) meets the need of stressed patients.

10 -Oxepa provides enough protein to promote anabolism and the maintenance of lean body mass without precipitating respiratory problems. High protein intakes are a concern in patients with respiratory insufficiency. Although protein has little effect on CO<sub>2</sub> production, a high protein diet will increase ventilatory drive.

15 -The protein sources of Oxepa are 86.8% sodium caseinate and 13.2% calcium caseinate.

- The amino acid profile of the protein system in Oxepa meets or surpasses the standard for high quality protein set by the National Academy of Sciences.

20

\* Oxepa is gluten-free.



Default settings for the analysis programs

## GCG Programs

5      FastA Search

Default parameters:

10      range of interest      Begin=1      END=last protein or nucleic  
acidsearch set      all of SwissProt (protein) or  
GenEMBL(nucleic acid)

15      word size      =(2) for protein      =(6) for nucleic acid

Expected scores lists scores until E( ) value reaches 2.0

20

TFastA search

Default parameters:

25      range of interest      Begin=1      END=last nucleic acid

search set      all of GenEMBL

30      word size      wordsize=(2)

Expected scores lists scores until E() value reaches 2.0

35

Pileup

Default parameters:

40      gap creation penalty      gap weight = 5

gap extension penalty      gap length weight = 12

plot figure      one page plot density =2.7

45

Sequencher Program

Default parameters:

50      Automatic Assembly      Dirty data algorithm =slower  
contig assembly but more

rigorous comparisons between the sequences

minimum match =85%

minimum overlap =20

## BLAST 2 (blastp, tblastn)

Default parameters: V=50 Lambda=.329 W=3  
B=50 K=0.140 X=22  
E=10 H=0.427

blast n

Default parameters: V=100 Lambda=1.37 W=11  
B=250 K=0.171 X1=22  
E=10 H=1.31 X2=25

## BLAST 2 Command Line Arguments

-v Hits number of best scores to show

-b Alignments number of best alignments to show

-e Expectation value (E) [Real] default = 10.0

-m Alignment view options: 0 = pairwise,  
1 = master-slave showing identities,  
2 = master-slave, no identities,  
3 = flat master-slave, show identities,  
4 = flat master-slave, no identities,  
5 = master-slave, no identities and blunt ends,  
6 = flat master-slave, no identities and blunt ends  
[Integer]  
default = 0

-F Filter query seq. (DUST with blastn, SEG with others) [T/F]  
default = T

-G Cost to open a gap (zero invokes default behavior) [Integer]  
default = 0

-E Cost to extend a gap (zero invokes default behavior) [Integer]

```

                                default = 0

-X X dropoff value for gapped alignment (in bits) (zero invokes
default behavior) [Integer]
5                                default = 0

-I Show GI's in deflines                                [T/F]
                                default = F

10 -q Penalty for a nucleotide mismatch                (blastn only) [Integer]
                                default = -3

-r Reward for a nucleotide match                        (blastn only) [Integer]
                                default = 1

15 -f Threshold for extending hits                      default if zero [Integer]
                                default = 0

20 -g Perform gapped alignment (not available with tblastx) [T/F]
                                default = T

-q Query Genetic code to use                            [Integer]
                                default = 1

25 -D DB Genetic code                                (for tblast[nx] only)
[Integer]
                                default = 1

30 -J Believe the query defline                        [T/F]
                                default = F

-M Matrix                                                [String]
                                default = BLOSUM62

35 -W Word size                                          default if zero [Integer]
                                default = 0

-z Effective length of the database (use zero for the real size)
[Integer]
40                                default = 0

-a Number of processors to use                        [Integer]
                                default = site configurable
                                (SeqServer.conf)
45

Allowed and default values for gap open/gap extension cost (-G/-
E) parameters:

50 BLOSUM62
-G          9      8      7      12     11     10

```

118

	-E	2	2	2	1	1	1					
	BLOSUM50											
5	-G	12	11	10	9	15	14	13	12	18	17	16
	-E	3	3	3	3	2	2	2	2	1	1	1
	PAM250											
10	-G	13	12	11	10	15	14	13	12	19	18	17
	-E	3	3	3	3	2	2	2	2	1	1	1
	BLOSUM90											
15	-G	8	7	6	11	10	9					
	-E	2	2	2	1	1	1					
	PAM30											
20	-G	5	4	3	7	6	5	10	9	8		
	-E	3	3	3	2	2	2	1	1	1		
	PAM70											
25	-G	6	5	4	8	7	6	11	10	9		
	-E	3	3	3	2	2	2	1	1	1		

CLAIMS:

1. An isolated nucleotide sequence corresponding to  
or complementary to at least about 50% of the  
5 nucleotide sequence shown in SEQ ID NO:1 (Figure 6).

2. The isolated nucleotide sequence of claim 1  
wherein said sequence is represented by SEQ ID NO:1.

10 3. The isolated nucleotide sequence of claims 1 or 2  
wherein said sequence encodes a functionally active  
elongase which utilizes a polyunsaturated fatty acid  
or a monounsaturated fatty acid as a substrate.

15 4. The nucleotide sequence of claim 1 wherein said  
sequence is derived from a fungus of the genus  
Mortierella.

20 5. The nucleotide sequence of claim 4 wherein said  
fungus is of the species alpina.

6. A purified protein encoded by said nucleotide  
sequence of claims 1 or 2.

25 7. A purified polypeptide which elongates  
polyunsaturated fatty acids or monounsaturated fatty  
acids and has at least about 50% amino acid similarity  
to the amino acid sequence of said purified protein of  
claim 6.

30 8. A method of producing an elongase enzyme  
comprising the steps of:

- a) isolating said nucleotide sequence represented by SEQ ID NO:1 (Figure 6);
- b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to  
5 ii) a promoter;
- c) introducing said vector into a host cell under time and conditions sufficient for expression of said elongase enzyme.

10 9. The method of claim 8 wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

15 10. The method of claim 9 wherein said prokaryotic cell is selected from the group consisting of E. coli, cyanobacteria, and B. subtilis.

20 11. The method of claim 9 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

25 12. The method of claim 11 wherein said fungal cell is selected from the group consisting of Saccharomyces spp., Candida spp., Lipomyces starkey, Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp.

30 13. The method of claim 11 wherein said fungal cell

is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp.

5 14. The method of claim 13 wherein said yeast cell is Saccharomyces cerevisiae.

15. A vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:1 (Figure 6) operably linked  
10 to b) a promoter.

16. A host cell comprising said vector of claim 15.

15 17. The host cell of claim 16, wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

18. The host cell of claim 17 wherein said prokaryotic cell is selected from the group consisting  
20 of E. coli, Cyanobacteria, and B. subtilis.

19. The host cell of claim 17 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and  
25 a fungal cell.

20. The host cell of claim 19 wherein said fungal cell is selected from the group consisting of Saccharomyces spp., Candida spp., Lipomyces starkey,  
30 Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp.

21. The host cell of claim 20 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp.,  
5 Hansenula spp. and Pichia spp.

22. The host cell of claim 21 wherein said host cell is Saccharomyces cerevisiae.

10 23. A plant cell, plant or plant tissue comprising said vector of claim 15, wherein expression of said nucleotide sequence of said vector results in production of at least one fatty acid selected from the group consisting of a monounsaturated fatty acid  
15 and a polyunsaturated fatty acid by said plant cell, plant or plant tissue.

24. The plant cell, plant or plant tissue of claim 23 wherein said polyunsaturated fatty acid is selected  
20 from the group consisting dihomog- $\gamma$ -linolenic acid (DGLA), 20:4n-3, and adrenic acid (ADA).

25 25. One or more plant oils or fatty acids expressed by said plant cell, plant or plant tissue of claim 23.

26. A transgenic plant comprising said vector of claim 15, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic  
30 plant.

27. A transgenic, non-human mammal whose genome



comprises a DNA sequence encoding an elongase operably linked to a promoter.

28. The transgenic, non-human mammal of claim 27,  
5 wherein said DNA sequence is represented by SEQ ID NO:1 (Figure 6).

29. A fluid produced by said transgenic, non-human  
10 mammal of claim 28 wherein said fluid comprises a detectable level of at least one elongase or products thereof.

30. A method for producing a polyunsaturated fatty acid comprising the steps of:

- 15 a) isolating said nucleotide sequence represented by SEQ ID NO:1 (Figure 6);  
b) constructing a vector comprising said isolated nucleotide sequence;  
c) introducing said vector into a host cell under time  
20 and conditions sufficient for expression of elongase enzyme encoded by said isolated nucleotide sequence; and  
d) exposing said expressed elongase enzyme to a  
25 substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

31. The method according to claim 30, wherein said  
30 substrate polyunsaturated fatty acid is selected from the group consisting of  $\gamma$ -linolenic acid (GLA), stearidonic acid (STA) and arachidonic acid (AA), and said product polyunsaturated fatty acid is selected

from the group consisting of DGLA, 20:4n-3, and ADA, respectively.

32. The method according to claim 30 further comprising the step of exposing said product polyunsaturated fatty acid to at least one desaturase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid.

33. The method according to claim 32 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, and ADA said another polyunsaturated fatty acid is selected from the group consisting of AA, eicosapentaenoic acid (EPA),  $\omega$ 6-docosapentaenoic acid, respectively, and said at least one desaturase is  $\Delta$ 5-desaturase, with respect to production of AA or EPA, and  $\Delta$ 4-desaturase, with respect to production of  $\omega$ 6-docosapentaenoic acid.

34. The method of claim 32 further comprising the step of exposing said another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert said another polyunsaturated fatty acid to a final polyunsaturated fatty acid.

35. The method of claim 34 wherein said final

polyunsaturated fatty acid is selected from the group consisting of docosahexaenoic acid (DHA), AA,  $\omega$ 6-docosapentaenoic acid, and  $\omega$ 3-docosapentaenoic acid.

5        36. A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 30, said  
10        another polyunsaturated fatty acid produced according to the method of claim 32, and said final polyunsaturated fatty acid produced according to the method of claim 34.

15        37. The nutritional composition of claim 36 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3 and ADA.

20        38. The nutritional composition of claim 36 wherein said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA, and  $\omega$ 6-docosapentaenoic acid.

25        39. The nutritional composition of claim 36 wherein said final polyunsaturated fatty acid is selected from the group consisting of DHA, adrenic acid,  $\omega$ 6-docosapentaenoic acid and  $\omega$ 3-docosapentaenoic acid.

30        40. The nutritional composition of claim 36 wherein said nutritional composition is selected from the group consisting of an infant formula, a dietary supplement and a dietary substitute.

41. The nutritional composition of claim 40 wherein said nutritional composition is administered to a human or an animal.

5

42. The nutritional composition of claim 41 wherein said nutritional composition is administered enterally or parenterally.

10

43. The nutritional composition of claim 40 wherein said nutritional composition further comprises at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, triglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, protein hydrolysates, sunflower oil, safflower oil, corn oil, and flax oil.

15

20

44. The nutritional composition of claim 43 wherein said nutritional composition further comprises at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.

25

30

45. A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 30,

said another polyunsaturated fatty acid produced according to the method of claim 32, and said final polyunsaturated fatty acid produced according to the method of claim 34 and 2) a pharmaceutically acceptable carrier.

46. The pharmaceutical composition of claim 45 wherein said pharmaceutical composition is administered to a human or an animal.

47. The pharmaceutical composition of claim 45 wherein said pharmaceutical composition further comprises at least one element selected from the group consisting of a vitamin, a mineral, a salt, a carbohydrate, an amino acid, a free fatty acid, a preservative, an excipient, an anti-histamine, a growth factor, an antibiotic, a diluent, a phospholipid, an antioxidant, and a phenolic compound.

48. An animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 30, said another polyunsaturated fatty acid produced according to the method of claim 32 and said final polyunsaturated fatty acid produced according to the method of claim 34.

49. The animal feed of claim 48 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, and ADA.

50. The animal feed of claim 48 wherein said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA, and  $\omega$ 6-docosapentaenoic acid.

5 51. The animal feed of claim 48 wherein said final polyunsaturated fatty acid is selected from the group consisting of DHA, ADA,  $\omega$ 6-docosapentaenoic acid and  $\omega$ 3-docosapentaenoic acid.

10 52. A cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 30, said another polyunsaturated fatty acid produced according to the method of claim 32 and  
15 said final polyunsaturated fatty acid produced according to the method of claim 34.

53. A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said  
20 nutritional composition of claim 36 in an amount sufficient to effect said prevention or treatment.

54. An isolated nucleotide sequence corresponding to  
25 or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO:2 (Figure 22).

55. The isolated nucleotide sequence of claim 54 wherein said sequence is represented by SEQ ID NO:2.

30 56. The isolated nucleotide sequence of claims 54 or 55 wherein said sequence encodes a functionally active

elongase which utilizes a polyunsaturated fatty acid as a substrate.

5 57. The nucleotide sequence of claim 55 wherein said sequence is derived from a fungus of the genus Mortierella.

10 58. The nucleotide sequence of claim 57 wherein said fungus is of the species alpina.

59. A purified protein encoded by said nucleotide sequence of claims 54 or 55.

15 60. A purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of said purified protein of claim 59.

20 61. A method of producing an elongase enzyme comprising the steps of:

- 25 a) isolating said nucleotide sequence represented by SEQ ID NO:2 (Figure 22);  
b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;  
c) introducing said vector into a host cell under time and conditions sufficient for expression of said elongase enzyme.

30 62. The method of claim 61 wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

63. The method of claim 62 wherein said prokaryotic cell is selected from the group consisting of E. coli, cyanobacteria, and B. subtilis.

5

64. The method of claim 62 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

10

65. The method of claim 64 wherein said fungal cell is selected from the group consisting of Saccharomyces spp., Candida spp., Lipomyces starkey, Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp.

15

66. The method of claim 65 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp.

20

67. The method of claim 66 wherein said yeast cell is Saccharomyces cerevisiae.

25

68. A vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:2 (Figure 22) operably linked to b) a promoter.

30

69. A host cell comprising said vector of claim 68.

70. The host cell of claim 69, wherein said host



cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

71. The host cell of claim 70 wherein said prokaryotic cell is selected from the group consisting of E. coli, Cyanobacteria, and B. subtilis.

72. The host cell of claim 70 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

73. The host cell of claim 72 wherein said fungal cell is selected from the group consisting of Saccharomyces spp., Candida spp., Lipomyces starkey, Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp.

74. The host cell of claim 73 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp.

75. The host cell of claim 74 wherein said host cell is Saccharomyces cerevisiae.

76. A plant cell, plant or plant tissue comprising said vector of claim 68, wherein expression of said nucleotide sequence of said vector results in

production of a polyunsaturated fatty acid by said plant cell, plant or plant tissue.

5 77. The plant cell, plant or plant tissue of claim 76 wherein said polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, and ADA.

78. One or more plant oils or acids expressed by said plant cell, plant or plant tissue of claim 76.

10 79. A transgenic plant comprising said vector of claim 68, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic plant.

15 80. A transgenic, non-human mammal whose genome comprises a DNA sequence, from M. alpina, encoding an elongase operably linked to a promoter.

20 81. The transgenic, non-human mammal of claim 80, wherein said DNA sequence is represented by SEQ ID NO:2 (Figure 22).

25 82. A fluid produced by said transgenic, non-human mammal of claim 81 wherein said fluid comprises a detectable level of at least one elongase or products thereof.

30 83. A method for producing a polyunsaturated fatty acid comprising the steps of:

- a) isolating said nucleotide sequence represented by  
SEQ ID NO:2 (Figure 22);
- b) constructing a vector comprising said isolated  
nucleotide sequence;
- 5 c) introducing said vector into a host cell under  
time and conditions sufficient for expression of  
an elongase enzyme encoded by said isolated  
nucleotide sequence; and
- 10 d) exposing said expressed elongase enzyme to a  
substrate polyunsaturated fatty acid in order to  
convert said substrate to a product  
polyunsaturated fatty acid.

15 84. The method according to claim 83, wherein said  
substrate polyunsaturated fatty acid is selected from  
the group consisting of GLA, STA, and AA, and said  
product polyunsaturated fatty acid is selected from  
the group consisting of DGLA, 20:4n-3, and  $\omega$ 6-  
docosapentaenoic acid, respectively.

20 85. The method according to claim 83 further  
comprising the step of exposing said expressed  
elongase enzyme to at least one desaturase in order to  
convert said product polyunsaturated fatty acid to  
25 another polyunsaturated fatty acid.

30 86. The method according to claim 85 wherein said  
product polyunsaturated fatty acid is selected from  
the group consisting of DGLA, 20:4n-3, and ADA said  
another polyunsaturated fatty acid is selected from  
the group consisting of AA, EPA, and  $\omega$ 6-  
docosapentaenoic acid, respectively, and said at least

one desaturase is  $\Delta 5$ -desaturase with respect to production of AA or EPA, and  $\Delta 4$ -desaturase with respect to production of  $\omega 6$ -docosapentaenoic acid.

5 87. The method of claim 86 further comprising the steps of exposing said another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert said another  
10 polyunsaturated fatty acid to a final polyunsaturated fatty acid.

88. The method of claim 87 wherein said final polyunsaturated fatty acid is selected from the group  
15 consisting of docosahexaenoic acid, AA,  $\omega 6$ -docosapentaenoic acid, and  $\omega 3$ -docosapentaenoic acid.

89. A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group  
20 consisting of said product polyunsaturated fatty acid produced according to the method of claim 83, said another polyunsaturated fatty acid produced according to the method of claim 85, and said final polyunsaturated fatty acid produced according to the  
25 method of claim 87.

90. The nutritional composition of claim 89 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3 and ADA.

30 91. The nutritional composition of claim 89 wherein

said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA, and  $\omega$ 6-docosapentaenoic acid.

5        92. The nutritional composition of claim 89 wherein said final polyunsaturated fatty acid is selected from the group consisting of DHA, AA,  $\omega$ 6-docosapentaenoic acid, and  $\omega$ 3-docosapentaenoic acid.

10       93. The nutritional composition of claim 89 wherein said nutritional composition is selected from the group consisting of an infant formula, a dietary supplement and a dietary substitute.

15       94. The nutritional composition of claim 93 wherein said nutritional composition is administered to a human or an animal.

20       95. The nutritional composition of claim 94 wherein said nutritional composition is administered enterally or parenterally.

25       96. The nutritional composition of claim 93 wherein said nutritional composition further comprises at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, triglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, protein  
30       hydrolysates, sunflower oil, safflower oil, corn oil, and flax oil.

97. The nutritional composition of claim 96 wherein said nutritional composition further comprises at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.

98. A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 83, said another polyunsaturated fatty acid produced according to the method of claim 85, and said final polyunsaturated fatty acid produced according to the method of claim 87 and 2) a pharmaceutically acceptable carrier.

99. The pharmaceutical composition of claim 98 wherein said pharmaceutical composition is administered to a human or an animal.

100. The pharmaceutical composition of claim 98 wherein said pharmaceutical composition further comprises an element selected from the group consisting of a vitamin, a mineral, a carbohydrate, an amino acid, a free fatty acid, a preservative, an excipient, an anti-histamine, a growth factor, an antibiotic, a phospholipid, an antioxidant, and a phenolic compound.

101. An animal feed comprising at least one  
polyunsaturated fatty acid selected from the group  
consisting of said product polyunsaturated fatty acid  
produced according to the method of claim 83, said  
5 another polyunsaturated fatty acid produced according  
to the method of claim 85 and said final  
polyunsaturated fatty acid produced according to the  
method of claim 87.

10 102. The animal feed of claim 101 wherein said  
product polyunsaturated fatty acid is selected from  
the group consisting of DGLA, 20:4n-3, and ADA.

15 103. The animal feed of claim 101 wherein said another  
polyunsaturated fatty acid is selected from the group  
consisting of AA, EPA, and  $\omega$ 6-docosapentaenoic acid.

20 104. The animal feed of claim 101 wherein said final  
polyunsaturated fatty acid is selected from the group  
consisting of DHA, adrenic acid,  $\omega$ 6-docosapentaenoic  
acid and  $\omega$ 3-docosapentaenoic acid.

25 105. A cosmetic comprising a polyunsaturated fatty  
acid selected from the group consisting of said  
product polyunsaturated fatty acid produced according  
to the method of claim 83, said another  
polyunsaturated fatty acid produced according to the  
method of claim 85 and said final polyunsaturated  
fatty acid produced according to the method of claim  
30 87.

106. A method of preventing or treating a condition  
caused by insufficient intake of polyunsaturated fatty  
acids comprising administering to said patient said  
nutritional composition of claim 89 in an amount  
5 sufficient to effect said treatment.

107. An isolated nucleotide sequence corresponding to  
or complementary to at least about 35% of the  
nucleotide sequence shown in SEQ ID NO:3 (Figure 43).  
10

108. The isolated nucleotide sequence of claim 107  
wherein said sequence is represented by SEQ ID NO:3.

109. The isolated nucleotide sequence of claims 107  
or 108 wherein said sequence encodes a functionally  
active elongase which utilizes a polyunsaturated fatty  
acid or a monounsaturated fatty acid as a substrate.  
15

110. The nucleotide sequence of claim 109 wherein  
said sequence is derived from a mammal.  
20

111. The nucleotide sequence of claim 110 wherein  
said mammal is a human.

112. A purified protein encoded by said nucleotide  
sequence of claims 107 or 108.  
25

113. A purified polypeptide which elongates  
polyunsaturated fatty acids or monounsaturated fatty  
acids and has at least about 30% amino acid similarity  
to the amino acid sequence of said purified protein of  
claim 112.  
30



114. A method of producing an elongase enzyme comprising the steps of:

- 5           a) isolating said nucleotide sequence represented by  
          SEQ ID NO:3 (Figure 43);
- b) constructing a vector comprising: i) said  
          isolated nucleotide sequence operably linked to  
          ii) a promoter;
- 10          c) introducing said vector into a host cell under  
          time and conditions sufficient for expression of  
          said elongase enzyme.

115. The method of claim 114 wherein said host cell  
15 is selected from the group consisting of a eukaryotic  
cell or a prokaryotic cell.

116. The method of claim 115 wherein said prokaryotic  
cell is selected from the group consisting of E. coli,  
cyanobacteria, and B. subtilis.

20 117. The method of claim 115 wherein said eukaryotic  
cell is selected from the group consisting of a  
mammalian cell, an insect cell, a plant cell and a  
fungal cell.

25 118. The method of claim 117 wherein said fungal cell  
is selected from the group consisting of Saccharomyces  
spp., Candida spp., Lipomyces starkey, Yarrowia spp.,  
Kluyveromyces spp., Hansenula spp., Aspergillus spp.,  
30 Penicillium spp., Neurospora spp., Trichoderma spp.  
and Pichia spp.

119. The method of claim 118 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp.

5

120. The method of claim 119 wherein said yeast cell is Saccharomyces cerevisiae.

10

121. A vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:3 (Figure 43) operably linked to b) a promoter.

122. A host cell comprising said vector of claim 121.

15

123. The host cell of claim 122, wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

20

124. The host cell of claim 123 wherein said prokaryotic cell is selected from the group consisting of E. coli, Cyanobacteria, and B. subtilis.

25

125. The host cell of claim 123 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

30

126. The host cell of claim 125 wherein said fungal cell is a selected from the group consisting of Saccharomyces spp., Candida spp., Lipomyces starkey, Yarrowia spp., Kluyveromyces spp., Hansenula spp.,

Aspergillus spp., Penicillium spp., Neurospora spp.,  
Trichoderma spp. and Pichia spp.

5 127. The host cell of claim 126 wherein said fungal  
cell is a yeast cell selected from the group  
consisting of Saccharomyces spp., Candida spp.,  
Hansenula spp. and Pichia spp.

10 128. The host cell of claim 127 wherein said host cell  
is Saccharomyces cerevisiae.

15 129. A plant cell, plant or plant tissue comprising  
said vector of claim 121, wherein expression of said  
nucleotide sequence of said vector results in  
production of at least one fatty acid selected from  
the group consisting of a monounsaturated fatty acid  
and a polyunsaturated fatty acid by said plant cell,  
plant or plant tissue.

20 130. The plant cell, plant or plant tissue of claim  
129 wherein said polyunsaturated fatty acid is  
selected from the group consisting of DGLA, 20:4n-3  
and ADA.

25 131. One or more plant oils or acids expressed by said  
plant cell, plant or plant tissue of claim 130.

30 132. A transgenic plant comprising said vector of  
claim 121, wherein expression of said nucleotide  
sequence of said vector results in production of a  
polyunsaturated fatty acid in seeds of said transgenic  
plant.

133. A transgenic, non-human mammal whose genome comprises a human DNA sequence encoding an elongase operably linked to a promoter.

5

134. The transgenic, non-human mammal of claim 133, wherein said DNA sequence is represented by SEQ ID NO:3 (Figure 43).

10

135. A fluid produced by said transgenic, non-human mammal of claim 134 wherein said fluid comprises a detectable level of at least one elongase or products thereof.

15

136. A method for producing a polyunsaturated fatty acid comprising the steps of:

- a) isolating said nucleotide sequence represented by SEQ ID NO:3 (Figure 43);
- b) constructing a vector comprising said isolated nucleotide sequence;
- c) introducing said vector into a host cell under time and conditions sufficient for expression of elongase enzyme encoded by said isolated nucleotide sequence; and
- d) exposing said expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

20

25

30

137. The method according to claim 136, wherein said substrate polyunsaturated fatty acid is selected from the group consisting of GLA, STA and AA, and said

product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, and ADA, respectively.

5 138. The method according to claim 136 further comprising the step of exposing said product polyunsaturated fatty acid to at least one desaturase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid.

10

139. The method according to claim 138 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3 and ADA, said another polyunsaturated fatty acid is selected from  
15 the group consisting of AA, EPA, and  $\omega$ 6-docosapentaenoic acid, respectively, and said at least one desaturase is  $\Delta$ 5-desaturase with respect to production of AA or EPA and  $\Delta$ 4-desaturase with respect to production of  $\omega$ 6-docosapentaenoic acid

20

140. The method of claim 138 further comprising the step of exposing said another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one  
25 additional desaturase in order to convert said another polyunsaturated fatty acid to a final polyunsaturated fatty acid.

141. The method of claim 140 wherein said final

polyunsaturated fatty acid is selected from the group consisting of DHA, ADA,  $\omega$ 6-docosapentaenoic acid, and  $\omega$ 3-docosapentaenoic acid.

5 142. A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 136, said  
10 another polyunsaturated fatty acid produced according to the method of claim 138, and said final polyunsaturated fatty acid produced according to the method of claim 140.

143. The nutritional composition of claim 142 wherein  
15 said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, and ADA.

144. The nutritional composition of claim 142 wherein  
20 said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA, and  $\omega$ 6-docosapentaenoic acid.

145. The nutritional composition of claim 142 wherein  
25 said final polyunsaturated fatty acid is selected from the group consisting of DHA, ADA,  $\omega$ 6-docosapentaenoic acid, and  $\omega$ 3-docosapentaenoic acid.

146. The nutritional composition of claim 142 wherein  
30 said nutritional composition is selected from the group consisting of an infant formula, a dietary supplement and a dietary substitute.

147. The nutritional composition of claim 146 wherein said nutritional composition is administered to a human or an animal.

5

148. The nutritional composition of claim 147 wherein said nutritional composition is administered enterally or parenterally.

10

149. The nutritional composition of claim 147 wherein said nutritional composition further comprises at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, triglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, protein hydrolysates, sunflower oil, safflower oil, corn oil, and flax oil.

15

20

150. The nutritional composition of claim 149 wherein said nutritional composition further comprises at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.

25

30

151. A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 136,

said another polyunsaturated fatty acid produced according to the method of claim 138, and said final polyunsaturated fatty acid produced according to the method of claim 140 and 2) a pharmaceutically acceptable carrier.

152. The pharmaceutical composition of claim 151 wherein said pharmaceutical composition is administered to a human or an animal.

153. The pharmaceutical composition of claim 151 wherein said pharmaceutical composition further comprises at least one element selected from the group consisting of a vitamin, a mineral, a salt, a carbohydrate, an amino acid, a free fatty acid, a preservative, an excipient, an anti-histamine, a growth factor, an antibiotic, a diluent, a phospholipid, an antioxidant, and a phenolic compound.

154. An animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 136, said another polyunsaturated fatty acid produced according to the method of claim 138 and said final polyunsaturated fatty acid produced according to the method of claim 140.

155. The animal feed of claim 154 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, and ADA.



156. The animal feed of claim 154 wherein said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA, and  $\omega$ 6-docosapentaenoic acid.

5 157. The animal feed of claim 154 wherein said final polyunsaturated fatty acid is selected from the group consisting of DHA, ADA,  $\omega$ 6-docosapentaenoic acid and  $\omega$ 3-docosapentaenoic acid.

10 158. A cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 136, said another polyunsaturated fatty acid produced according to the method of claim 138 and said final polyunsaturated fatty acid produced according to the method of claim 140.

15 20 159. A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said nutritional composition of claim 142 in an amount sufficient to effect said treatment.

25 160. An isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO:4 (Figure 46).

30 161. The isolated nucleotide sequence of claim 160 wherein said sequence is represented by SEQ ID NO:4.

162. The isolated nucleotide sequence of claims 160 or 161 wherein said sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate.

5

163. The nucleotide sequence of claim 162 wherein said sequence is derived from a nematode of the genus Caenorhabditis.

10

164. The nucleotide sequence of claim 163 wherein said nematode is of the species elegans.

165. A purified protein encoded by said nucleotide sequence of claims 160 or 161.

15

166. A purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of said purified protein of claim 165.

20

167. A method of producing an elongase enzyme comprising the steps of:

a) isolating said nucleotide sequence represented by SEQ ID NO:4 (Figure 46);

25

b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;

c) introducing said vector into a host cell under time and conditions sufficient for expression of said elongase enzyme.

30

168. The method of claim 167 wherein said host cell is

selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

5 169. The method of claim 168 wherein said prokaryotic cell is selected from the group consisting of E. coli, cyanobacteria, and B. subtilis.

10 170. The method of claim 168 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

15 171. The method of claim 170 wherein said fungal cell is selected from the group consisting of Saccharomyces spp., Candida spp., Lipomyces starkey, Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp.

20 172. The method of claim 171 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp.

25 173. The method of claim 172 wherein said yeast cell is Saccharomyces cerevisiae.

30 174. A vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:4 (Figure 46) operably linked to b) a promoter.

175. A host cell comprising said vector of claim 174.

176. The host cell of claim 175, wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

5

177. The host cell of claim 176 wherein said prokaryotic cell is selected from the group consisting of E. coli, Cyanobacteria, and B. subtilis.

10

178. The host cell of claim 176 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

15

179. The host cell of claim 178 wherein said fungal cell is selected from the group consisting of Saccharomyces spp., Candida spp., Lipomyces starkey, Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp.

20

180. The host cell of claim 179 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp.

25

181. The host cell of claim 180 wherein said host cell is Saccharomyces cerevisiae.

30

182. A plant cell, plant or plant tissue comprising said vector of claim 174, wherein expression of said nucleotide sequence of said vector results in

production of a polyunsaturated fatty acid by said plant cell, plant or plant tissue.

183. The plant cell, plant or plant tissue of claim 5 182 wherein said polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, and ADA.

184. One or more plant oils or acids expressed by said 10 plant cell, plant or plant tissue of claim 182.

185. A transgenic plant comprising said vector of claim 174, wherein expression of said nucleotide 15 sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic plant.

186. A transgenic, non-human mammal whose genome 20 comprises a DNA sequence, from C. elegans, encoding an elongase operably linked to a promoter.

187. The transgenic, non-human mammal of claim 186, 25 wherein said DNA sequence is represented by SEQ ID NO:4 (Figure 46).

188. A fluid produced by said transgenic, non-human 30 mammal of claim 187 wherein said fluid comprises a detectable level of at least one elongase or products thereof.

189. A method for producing a polyunsaturated fatty acid comprising the steps of:

- a) isolating said nucleotide sequence represented by  
SEQ ID NO:4 (Figure 46);
- b) constructing a vector comprising said isolated  
nucleotide sequence;
- 5 c) introducing said vector into a host cell under  
time and conditions sufficient for expression of  
an elongase enzyme encoded by said isolated  
nucleotide sequence; and
- 10 d) exposing said expressed elongase enzyme to a  
substrate polyunsaturated fatty acid in order to  
convert said substrate to a product  
polyunsaturated fatty acid.

15 190. The method according to claim 189, wherein said  
substrate polyunsaturated fatty acid is selected from  
the group consisting of GLA, STA, and AA and said  
product polyunsaturated fatty acid is DGLA, 20:4n-3,  
and ADA, respectively.

20 191. The method according to claim 189 further  
comprising the step of exposing said expressed  
elongase enzyme to at least one desaturase in order to  
convert said product polyunsaturated fatty acid to  
another polyunsaturated fatty acid.

25 192. The method according to claim 191 wherein said  
product polyunsaturated fatty acid is selected from  
the group consisting of DGLA, 20:4n-3 and ADA, said  
another polyunsaturated fatty acid is selected from  
30 the group consisting of AA, EPA and  $\omega$ 6-  
docosapentaenoic acid, respectively, and said at least  
one desaturase is  $\Delta$ 5-desaturase with respect to

production of AA or EPA, and  $\Delta 4$ -desaturase with respect to production of  $\omega 6$ -docosapentaenoic acid.

5 193. The method of claim 192 further comprising the steps of exposing said another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert said another polyunsaturated fatty acid to a final polyunsaturated fatty acid.

10 194. The method of claim 193 wherein said final polyunsaturated fatty acid is selected from the group consisting of DHA, ADA,  $\omega 6$ -docosapentaenoic acid, and  $\omega 3$ -docosapentaenoic acid.

15 195. A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 189, said another polyunsaturated fatty acid produced according to the method of claim 191, and said final polyunsaturated fatty acid produced according to the method of claim 193.

20 196. The nutritional composition of claim 195 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, and ADA.

25 197. The nutritional composition of claim 195 wherein

said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA, and  $\omega$ 6-docosapentaenoic acid.

5        198. The nutritional composition of claim 195 wherein said final polyunsaturated fatty acid is selected from the group consisting of DHA, ADA,  $\omega$ 6-docosapentaenoic acid, and  $\omega$ 3-docosapentaenoic acid.

10       199. The nutritional composition of claim 195 wherein said nutritional composition is selected from the group consisting of an infant formula, a dietary supplement and a dietary substitute.

15       200. The nutritional composition of claim 199 wherein said nutritional composition is administered to a human or an animal.

20       201. The nutritional composition of claim 200 wherein said nutritional composition is administered enterally or parenterally.

25       202. The nutritional composition of claim 199 wherein said nutritional composition further comprises at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, triglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, protein  
30       hydrolysates, sunflower oil, safflower oil, corn oil and flax oil.



203. The nutritional composition of claim 202 wherein said nutritional composition further comprises at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.

204. A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 189, said another polyunsaturated fatty acid produced according to the method of claim 191, and said final polyunsaturated fatty acid produced according to the method of claim 193 and 2) a pharmaceutically acceptable carrier.

205. The pharmaceutical composition of claim 204 wherein said pharmaceutical composition is administered to a human or an animal.

206. The pharmaceutical composition of claim 203 wherein said pharmaceutical composition further comprises an element selected from the group consisting of a vitamin, a mineral, a carbohydrate, an amino acid, a free fatty acid, a preservative, an excipient, an anti-histamine, a growth factor, an antibiotic, a phospholipid, an antioxidant, and a phenolic compound.

207. An animal feed comprising at least one  
polyunsaturated fatty acid selected from the group  
consisting of said product polyunsaturated fatty acid  
produced according to the method of claim 189, said  
5 another polyunsaturated fatty acid produced according  
to the method of claim 191 and said final  
polyunsaturated fatty acid produced according to the  
method of claim 193.

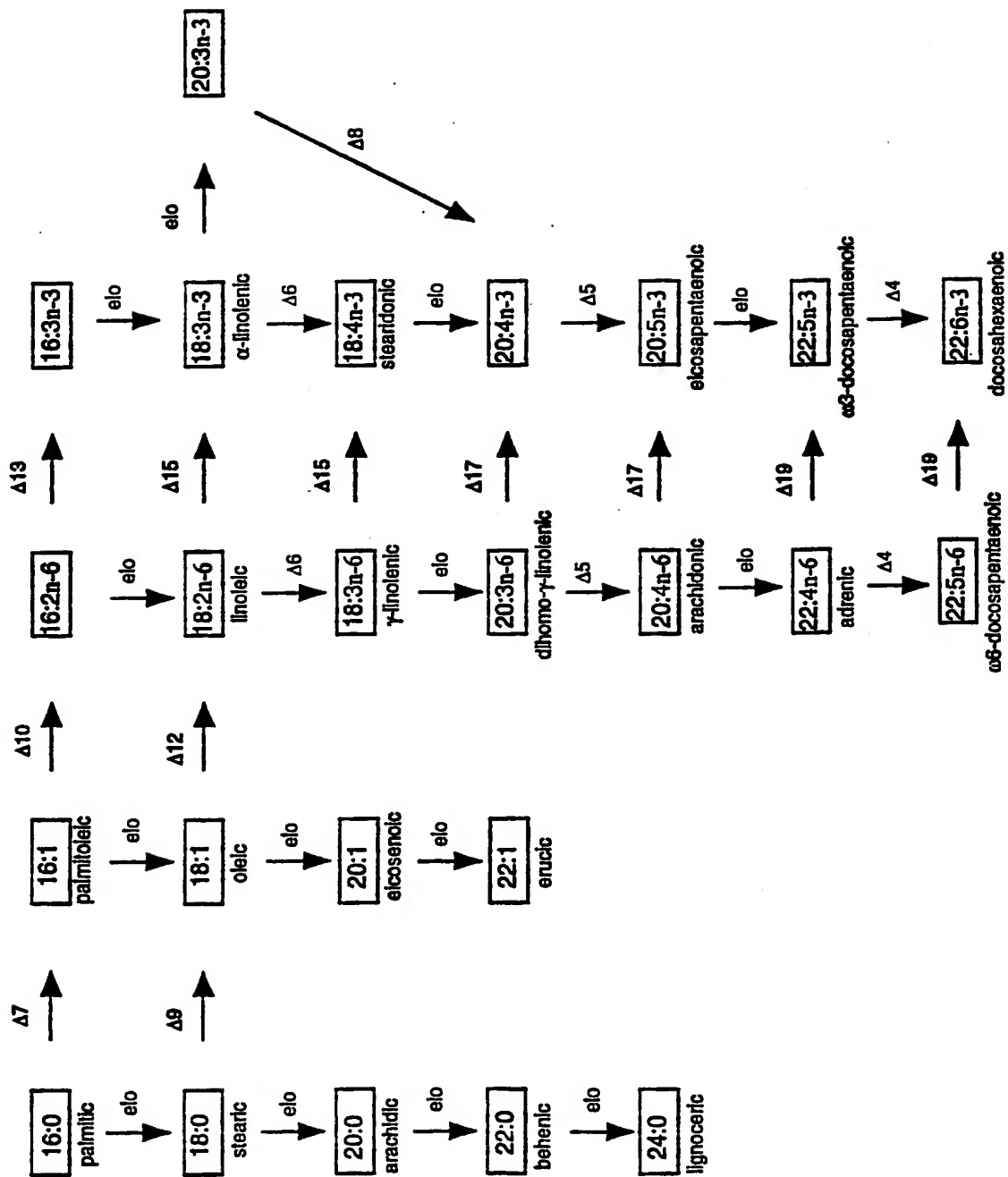
10 208. The animal feed of claim 207 wherein said  
product polyunsaturated fatty acid is selected from  
the group consisting of DGLA, 20:4n-3 and ADA.

15 209. The animal feed of claim 207 wherein said another  
polyunsaturated fatty acid is selected from the group  
consisting of AA, EPA and  $\omega$ 6-docosapentaenoic acid.

20 210. The animal feed of claim 207 wherein said final  
polyunsaturated fatty acid is selected from the group  
consisting of DHA, ADA,  $\omega$ 6-docosapentaenoic acid and  
 $\omega$ 3-docosapentaenoic acid.

25 211. A cosmetic comprising a polyunsaturated fatty  
acid selected from the group consisting of said  
product polyunsaturated fatty acid produced according  
to the method of claim 189, said another  
polyunsaturated fatty acid produced according to the  
method of claim 191 and said final polyunsaturated  
fatty acid produced according to the method of claim  
30 193.

212. A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said nutritional composition of claim 195 in an amount  
5 sufficient to effect said prevention or treatment.

**Figure 1****Fatty Acid Biosynthesis Pathways**

## Figure 2

Gap Weight: 6      Average Match: 2.912  
 Length Weight: 4      Average Mismatch: -2.003

Quality: 50      Length: 84  
 Ratio: 0.625      Gaps: 4  
 Percent Similarity: 43.038      Percent Identity: 29.114

Match display thresholds for the alignment(s):

| = IDENTITY  
 : = 2  
 . = 1

jojobakcs x ELO2

June 4, 1998 08:23 ..

```
jojobakcs 24 ATLPNFKSSINLHHVKL.GYHYLISNALFLVFIPLLGLASAHLSFSFSAHD 72
          .||| .| ::| :| :||| . | || | ..
ELO2      66 STLPPVLYAITAYYVIIFGGRFLLSKS..KPF.KLNGLFQLHNLVLTSL 112
```

```
jojobakcs 73 LSLFDLLRRNLLPVVVCSEFLVLLATLHFLTRP 106
          |.|| |:|.||:| |: : :|.|
ELO2      113 LTLL.LMVEQLVPITVQHGLYFAICNIGAWTQP 145
```

3/51

Figure 3

*S. cerevisiae* ELO2 (AA66-145) with *M. alpina* codon bias

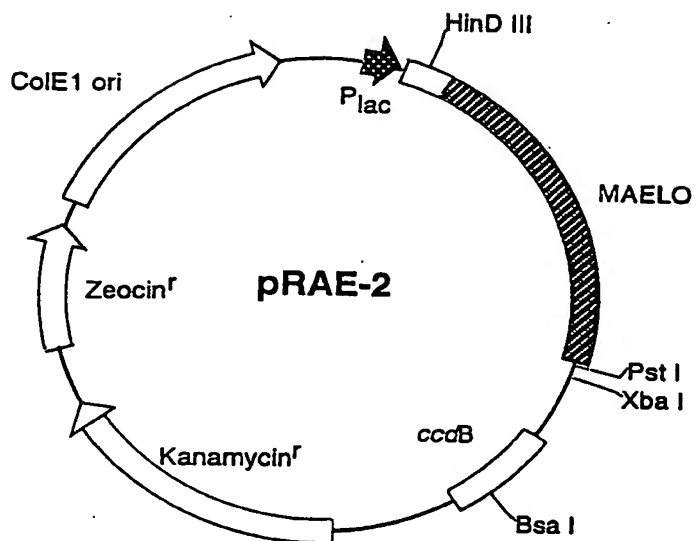
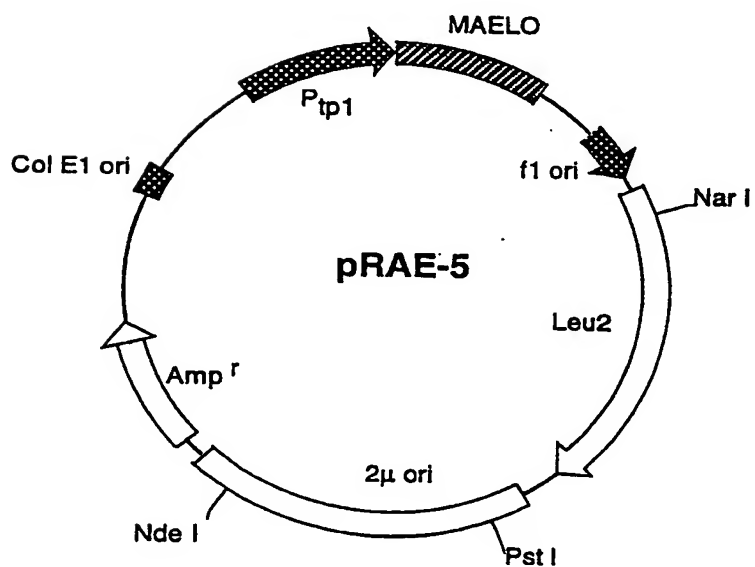
S	T	L	P	P	P	V	L	Y	A	I	T	A	Y	Y	V	I	I	F	G	G	R	F	L
TCC	ACC	CTC	CCC	CCC	CCC	GTC	CTC	TAC	GCC	ATC	ACC	GCC	TAC	TAC	GTC	ATC	ATC	TTC	GGT	GGT	CGC	TTC	CTC
66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	

<--- RO339

L	S	K	AAG	TCC	AAG	CCC	TTC	AAG	CTC	AAC	GGT	CTC	TTC	CAG	CTC	CAC	AAC	CTC	GTC	CTC	ACC	TCC	CTC
89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	

S	L	T	L	L	L	L	M	V	E	Q	L	V	P	I	I	V	Q	H	G	L	Y	F
TCC	CTC	ACC	CTC	CTC	CTC	ATG	GTC	GAG	CAG	CTC	GTC	CCC	ATC	ATC	GTC	CAG	CAC	GGT	CTC	TAC	TTC	
112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134

A	I	C	N	I	G	A	W	T	Q	P
GCC	ATC	TGC	AAC	ATC	GGT	GCC	TGG	ACC	CAG	CCC
135	136	137	138	139	140	141	142	143	144	145

**Figure 4****A****B**





**Figure 6**

```
1  ATGGCCGCCG CAATCTTGGA CAAGGTCAAC TTCGGCATTG ATCAGCCCTT
51  CGGAATCAAG CTCGACACCT ACTTTGCTCA GGCCTATGAA CTCGTCACCG
101 GAAAGTCCAT CGACTCCTTC GTCTTCCAGG AGGGCGTCAC GCCTCTCTCG
151 ACCCAGAGAG AGGTCGCCAT GTGGACTATC ACTTACTTCG TCGTCATCTT
201 TGGTGGTCGC CAGATCATGA AGAGCCAGGA CGCCTTCAAG CTCAAGCCCC
251 TCTTCATCCT CCACAACCTC CTCCTGACGA TCGCGTCCGG ATCGCTGTTG
301 CTCCTGTTCA TCGAGAACCT GGTCCCCATC CTCGCCAGAA ACGGACTTTT
351 CTACGCCATC TGCACGACG GTGCCTGGAC CCAGCGCCTC GAGCTCCTCT
401 ACTACCTCAA CTACCTGGTC AAGTACTGGG AGTTGGCCGA CACCGTCTTT
451 TTGGTCCTCA AGAAGAAGCC TCTTGAGTTC CTGCACTACT TCCACCACTC
501 GATGACCATG GTTCTCTGCT TTGTCCAGCT TGGAGGATAC ACTTCAGTGT
551 CCTGGGTCCC TATTACCTC AACTTGACTG TCCACGTCTT CATGTACTAC
601 TACTACATGC GCTCCGCTGC CGGTGTTTCG ATCTGGTGGA AGCAGTACTT
651 GACCACTCTC CAGATCGTCC AGTTCGTTCT TGACCTCGGA TTCATCTACT
701 TCTGCGCCTA CACCTACTTC GCCTTCACCT ACTTCCCCTG GGCTCCCAAC
751 GTCGGCAAGT GCGCCGGTAC CGAGGGTGCT GCTCTCTTTG GCTGCGGACT
801 CCTCTCCAGC TATCTCTTGC TCTTTATCAA CTTCTACCGC ATTACCTACA
851 ATGCCAAGGC CAAGGCAGCC AAGGAGCGTG GAAGCAACTT TACCCCCAAG
901 ACTGTCAAGT CCGGCGGATC GCCCAAGAAG CCCTCCAAGA GCAAGCACAT
951 CTAA
```

**Figure 7**

1 MAAAILDKVN FGIDQPFGIK LDTYFAQAYE LVTGKSIDSF VFQEGVTPLS  
51 TQREVAMWTI TYFVVIFGGR QIMKSQDAFK LKPLFILHNF LLTIASGSL  
101 LLFIENLVPI LARNGLFYAI CDDGAWTQRL ELLYYLNYLV KYWELADTVF  
151 LVLKKKPLEF LHYFHHMTM VLCFVQLGGY TSVSWVPITL NLTVHVFMYY  
201 YYMRSAAGVR IWWKQYLTTL QIVQFVLDLG FIYFCAYTYF AFTYFPWAPN  
251 VGKCAGTEGA ALFGCGLLSS YLLLFINFYR ITYNAKAKAA KERGSNFTPK  
301 TVKSGGSPKK PSKSKHI\*



9/51

Figure 9

SCORES Init1: 153 Inith: 199 Opt: 495  
57.4% identity in 549 bp overlap

```

      150      160      170      180      190      200
MAELO  TCTCGACCCAGAGAGAGGTCGCCATGTGGACTATCACTTACTTCGTGTCATCTTTGGTG
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
S78624 CATTAAACACTTTGCCCCCTGTGCTATACGCCATCACTGCCTATTACGTTATTATTTTGG
5990      6000      6010      6020      6030      6040

      210      220      230      240      250      260
MAELO  GTGCCAGATCATGAAGAGCCAG--GACGCC--TTCAAGCTCAACCCCTCTTCATCCTCC
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
S78624 GTGGCAGGTTTGTGTTAAGTAAGTCGAAACCATTTAAATTAAATGGCCTTTTCCAATTGC
6050      6060      6070      6080      6090      6100

      270      280      290      300      310      320
MAELO  ACRACCTTCCTCGACGATCGCGTCC--GGATCGCTGTTGCTCCTGTTTCATCGAGAACCT
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
S78624 ATAATTTGGTTTAAAC--TTCACCTTTCATTGA--CGCTTTTATGCTTATGGTTGAACAATT
6110      6120      6130      6140      6150      6160

      330      340      350      360      370      380
MAELO  GGTCCCATCTCGCCAGAAACGGACTTTTCTACGCCATCTGCGACGACGGTGCCTGGAC
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
S78624 AGTGCCAAATTATTGTTTTCAGCACGGGTTATCTTCTGCTATCTGTAATATTGGTGGTGGAC
6170      6180      6190      6200      6210      6220

      390      400      410      420      430      440
MAELO  CCAGCGCCTCGAGCTCCTCTACTACCTCAACTACCTGGTCAAGTACTGGGAGTTGGCCGA
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
S78624 TCAACCGCTCGTTACATTATATATACATGAATTACATTGTCAAGTTATTGAATTTATAGA
6230      6240      6250      6260      6270      6280

      450      460      470      480      490      500
MAELO  CACCGTCTTTTGGTCTCAAGAAGAAGCCTCTTGAGTTCCTGCACTACTTCCACCACTC
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
S78624 CACCTTTTCTTGGTGCTAAACATAAAAAATTGACATTTTGTCA--TACTT--ATCA--C
6290      6300      6310      6320      6330      6340

      510      520      530      540      550
MAELO  GATGACCATGGTCTCTGCTTTGT----CCAGCTTGGAGGATA--CACTTCAGTGTCTCTGG
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
S78624 CATGGCGCTACTGCCTTATTATGTTACACCCAATTGATGGGCACCACATCTATTTCTTGG
6350      6360      6370      6380      6390      6400

      560      570      580      590      600      610
MAELO  GTCCCTATTACCTCAACTTGACTGTCCAGCTCTTCATGTACTACTACTACATGGGCTCC
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
S78624 GTCCCTATTTCATTGAACCTTGGTGTTCACGTGGTTATGTATTGGTACTATT--CTTG
6410      6420      6430      6440      6450

      620      630      640      650      660      670
MAELO  GCTGCC---GGTGTTCGCATCTGGTGGAGCAGTACTTGACCACTCTCCAGATCGTCCAG
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
S78624 GCTGCCAGAGGCATCAGGGTCTGGTGGAGGAATGGGTACCAGATTTCAAATTATCCAA
6460      6470      6480      6490      6500      6510

      680      690      700      710      720      730
MAELO  TTCGTTCTTGACCTCGGATTTCATCTACTTCTGCGCCTACACCTACTTCGCCTTCACCTAC
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
S78624 TTTGTTTTGGATATCGGTTTCATATATTTTGTCTGTCTACCAAAAAGCAGTTCACTTGTAT
6520      6530      6540      6550      6560      6570
```

Figure 10A

Host(plasmid)	334(pCGN7875)	334(pYES2)	334(pYX242)	334(pRAE-5)	334(pRAE-6)	334(pYX242)	334(pRAE-5)
Added substrate	25 $\mu$ M OA	25 $\mu$ M OA	25 $\mu$ M GLA	25 $\mu$ M GLA	25 $\mu$ M GLA	no substrate	no substrate
Fatty acid	lipid ( $\mu$ g)	lipid ( $\mu$ g)	lipid ( $\mu$ g)	lipid ( $\mu$ g)	lipid ( $\mu$ g)	lipid ( $\mu$ g)	lipid ( $\mu$ g)
C16:0	11.948	23.601	35.123	92.011	85.160	16.294	25.34
C16:1	30.665	71.217	32.789	315.464	115.456	56.183	113.913
C18:0	6.185	9.704	10.515	22.628	18.879	5.535	11.092
C18:1n-9	35.340	57.429	33.989	154.386	106.881	28.388	51.538
C18:3n-6			48.856	58.084	12.434		
C20:0			0.474	0.710	0.244		
C20:1n-9	(0.375%)* 0.352	(0.309%)* 0.527		1.405	0.867		0.516
C20:3n-6	ND	ND	(0.092%)* 0.226	(0.324%)* 2.504	(0.269%)* 1.006	ND	ND
C22:0				0.460			
C22:1n-9				0.321	0.315		
C24:0					1.825		0.999
Total Lipid	93.760	170.490	245.090	771.690	374.420	112.99	256.52
ND = Not Detected							
*% total fatty acid							

Figure 10B

Host(plasmid)	334(pYX242)	334(pYX242)	334(pRAE-5)	334(pRAE-5)	334(pRAE-6)
Added substrate	25 $\mu$ M GLA	25 $\mu$ M GLA	25 $\mu$ M GLA	25 $\mu$ M GLA	25 $\mu$ M GLA
Fatty acid	lipid ( $\mu$ g)	lipid ( $\mu$ g)	lipid ( $\mu$ g)	lipid ( $\mu$ g)	lipid ( $\mu$ g)
C16:0	60.683	61.487	100.998	96.193	66.761
C16:1	79.838	79.586	359.754	220.440	87.359
C18:0	9.784	10.106	15.317	15.165	16.744
C18:1n-9	38.536	39.936	108.472	89.637	71.631
C18:3n-6	17.974	17.833	82.866	56.596	17.766
C20:0			0.510	0.570	
C20:1n-9					
C20:3n-6	(0.136%)* 0.389	(0.130%)* 0.374	(0.336%)* 3.035	(0.401%)* 2.689	(0.353%)* 1.185
C22:0			0.414		
C22:1n-9				0.383	
C24:0			1.513	1.626	
Total Lipid	285.560	288.045	902.560	671.113	335.496
*% total fatty acid					

12/51

Figure 11

Host(plasmid)	334(pRAB-5/pCGR4)	334(pYX242/pYES2)	Host(plasmid)	334(pRAB-5/pCGR4)	334(pYX242/pYES2)
Added substrate	25 $\mu$ M GLA	25 $\mu$ M GLA	Added substrate	25 $\mu$ M GLA	25 $\mu$ M GLA
Fatty Acid	lipid ( $\mu$ g)	lipid ( $\mu$ g)		lipid ( $\mu$ g)	lipid ( $\mu$ g)
C16:0	41.050	37.169	C16:0	96.986	32.221
C16:1	99.393	100.552	C16:1n-7	209.667	62.757
C18:0	34.432	27.852	C18:0	80.418	14.027
C18:1	110.631	92.786	C18:1n-9	207.104	28.701
C18:3n-6	15.004	7.924	C18:3n-6	25.264	10.543
C20:0	0.643	0.574	C20:0	2.038	
C20:1	1.996	1.684	C20:1n-9	3.591	
C20:3n-6	0.542	0.607	C20:3n-6	1.284	0.326
C20:4n-6	0.579		C20:4n-6	1.392	
C22:0	1.242	2.604	C22:0	1.124	
C24:0	4.754	4.563	C24:0	3.952	
Total Lipid	334	300	Total Lipid	756	197

Figure 12

Host(plasmid)	334(pYX242)	334(pRAE-5)	334(pRELO-1)	334(pRELO-2)
Added substrate	25 $\mu$ M GLA	25 $\mu$ M GLA	25 $\mu$ M GLA	25 $\mu$ M GLA
	25°C/48hrs	25°C/48hrs	25°C/48hrs	25°C/48hrs
Fatty acid	lipid ( $\mu$ g)	lipid ( $\mu$ g)	lipid ( $\mu$ g)	lipid ( $\mu$ g)
C16:0	28.7	76.707	84.424	77.445
C16:1	0.729	2.513	1.532	1.056
C18:0	7.432	15.761	27.17	21.32
C18:1n-9	28.9	77.323	109.419	82.844
C18:3n-6	9.729	29.236	19.085	18.804
C20:0		0.643	0.522	0.537
C20:1n-9		0.77	0.426	0.299
C20:3n-6	(0.185%)* 0.374	(0.279%)* 1.472	(0.153%)* 0.748	(0.200%)* 0.832
C22:0		0.451		
C22:1n-9			0.224	
C24:0		0.918		
Total Lipid	202	527	490	416
*%total fatty acid				



14/51

## Figure 13

SCORES Init1: 156 Initn: 215 Opt: 296  
 Smith-Waterman score: 296; 28.8% identity in 264 aa overlap

	10	20	30	40	50	60	
U61954	RTFKMMDQILGTNFTYEGAKEVARGLEGFSAKLAVGYIATIFGLKYMKDRKAFDLSTPL						
MAELO	AQAYELVTGKSIDSFVFOEGVTPLESTQREVAMWTITYFVVIFGGRQIMKSQDAFKLK-PL						
	30	40	50	60	70	80	
	70	80	90	100	110	119	120
U61954	NIWNGILSTFSLGFLTF-PTLLSVIRKDGFSHTYSHVSELYTDSTSGYWI-----F						
MAELO	FILHNFLLTIASGSLLLLFIEENLVPILARNGL-----FYAICDDGAWTORLELLYY						
	90	100	110		120	130	
	130	140	150	160	170		
U61954	LWVISKIPELLDTVFIVLRKRPLIFMHWYHHALTGYYALVCYHE--DAVHMVWV-VWMNY						
MAELO	LNYLVKYWELADTVFLVLKKKPLEFLHYFHHSMT---MVLCFVQLGGYTSVSWVPITLNL						
	140	150	160	170	180	190	
	180	190	200	210	220	230	240
U61954	IIHAFMYGYLLKSLKVPIPPSVAQAITSQMVQFA-----VAIFAQVHVSYPHYVEGVE						
MAELO	TVHVFMYYYMRSAAGVRI--WWKQYLTLQIVQFVLDLGFIFYFCAYTYFAFTYFPWAPN						
	200	210	220	230	240	250	
	240	250	260	270	280		
U61954	-GLAYSFRGTAI-GFFMLTTYFYLWIQFYKEHYLKNGGKKYNLAKDQAKTQTKKAN						
MAELO	VGKCACTEGAALFGCGLLSYLLLFINFYRITY----NAKAKAAKERGSNFTPKTVKSGG						
	260	270	280	290	300		
MAELO	SPKKPSKSKHIX						
	310						

15/51

Figure 14

```

268749      50      60      70      80      90      100
SLLTNQDEVFPHIRARRFIQEHFGLFVQMAIAYVILVFSIKRFMRDREPFQLTTLALRWLN
|:|::|:|::|::|:|:|
MAELO      ELVTGKSIDSFVFQEGVTPLPSTQREVAMWTITYFVVIFGGROIMKSQDAFKLKPLFILHN
30      40      50      60      70      80

      110      120      130      140      150      160
268749      FFLSVFSIYGSWTMFPF--MVQQIRLYGLYGCCEALSNLPSQAEYWLFLTILSKAVEFV
|:|::| | |:::| |::|:|::| |::| |::| |::| |::|
MAELO      FLTLIAS--GSLLLLFIEINLVPILARNGLFYAICDD-GAWTORLELLYYLNYLVKYWELA
90      100      110      120      130      140

      170      180      190      200      210      220
268749      DTFFVLVLKPKPLIFLHWHYHMMATFVFFCSNYPTPSSQSRVGVIVNLFVHAFMYPYFTR
| | | | |::| | |::| |::| |::| |::| |::| |::| |::| |::|
MAELO      DTVFVLVLKPKPLEFLHYFHHSMTMVLCFVQLGGYTSVSWVPITLNLTVHVFMYYYMRS
150      160      170      180      190      200

      230      240      250      260      270
268749      MNIKVPAKISMAVTVLQLTQF---MCFIYGCTLMYSLATNQARYPSNTPATLQCLSYTL
:::| |::| |::| |::| |::| |::| |::|
MAELO      AGVRIWWK--QYLTTLQIVQFVLDLGFIFYCAYTYFAFTYFFWAPNVGKCACTEGAALFG
210      220      230      240      250      260

280
268749      HLL

MAELO      CGLLSSYLLLFINFYRITYNAKAKAAKERGSNFTPKTVKSGGSPKKPKSKKHIX
270      280      290      300      310

```

## Figure 15

SCORES      Init1:    30   Initn:    30   Opt:      40  
Smith-Waterman score: 49;      22.1% identity in 86 aa overlap

```

                                10      20      30      40
AF003134      MLYSITRRCYTFFVTSLHFYQLYVTECLENVIFNVLVNGQSINSRWKD
                |:::  |:::  ::::  |:  ::::  :
MAELO      MAAAILDKVNEFGIDQPFGIKLDTYFAQA---YELVTGKSIDSFVFQEGVT--PLSTQREV
                10      20      30      40      50

50      60      70      80      90      100
AF003134      AEKTITSFPPHF-----PQTFQOPHILTLHFLFFVFSVTLVTVFKKPKCEFPHSLA
                ||||  |  |  |  ::  ||  :::::  |:  :|
MAELO      AMWTITYFVVIFGGRQIMKSQDAFKLKPLFILHNPLLTIASGSLLLLFIENTLVPILARNG
                60      70      80      90      100      110
```

17/51

Figure 16

**Mouse**

SCORES Init1: 161 Initn: 191 Opt: 325  
 Smith-Waterman score: 325; 28.8% identity in 285 aa overlap

```

      10      20      30      39      40
U97107      MDTSMNFSRGLKMD--LMQPYDFETFDLRLPFLEEYWVSSF-----LIVV
      |::| : | |:: | :: | : : | : : : : :
MAELO      MAAAILDKVNFIDQPFGIKLDTYFAQAYELVTGKSIDSFVFOEGVTPLSTQREVAMWTI
      10      20      30      40      50      60

      50      60      70      80      90      100
U97107      VYLLLIIVVGQTYMTRKSFSLQRLILWSFELAIIFSILGLRMWKFMATVMETVGLKQTV
      :|::| | : |:: :|:: :|| :|::| | | : | : | : : : : : : : :
MAELO      TYFVVIFFGGRQIMKSQDAFKLPLFILHNFILTIAS--GSL-LLLFIENLV--PILARNGL
      70      80      90      100      110

      110      120      130      140      150
U97107      CFAIYTDDAVVFWSFLELLSKV---ELGDTAFIILRKRELIFFVHWYHHST--VLLFTS
      :| | : : | : : | |::|::|::|::| | : : : | | : :
MAELO      FYAICDDGAWTQRLLELYYLNVLVKYWEADTVFLVLKKKPLEFLHYFHHSMIMVLCTVQ
      120      130      140      150      160      170

      160      170      180      190      200      210
U97107      FGYKNKVPSSGGWF-MTMNFGVHSVMTYYTMTKAAKLKHPNLLPMVITSIQILQMVLG---
      :| : : | : : : | | | | : | : : : : : : : : : : : :
MAELO      LGGYTSV---SWVPITLNLTVHVFMYYYYMRSAGVR--IWWKQYLTTLQIVQFVLDLGF
      180      190      200      210      220      230

      220      230      240      250      260
U97107      -----TIFGIILNIWRQEGK-CHTTTEHFFWSEMLYGTIFILFAHFFHRAYLRFKQKVA
      | : : : | : : | | : : | : : : | : : : | : : : | : : : |
MAELO      IYFCAYTYFAFTYFPWAPNVGKCGAGTEGAALFGCGLLSSYLLLFNFYRITY-NAKAKAA
      240      250      260      270      280      290

      270
U97107      SKSQX
      : :
MAELO      KERGSNFTPKTVKSGGSPKKPSKSKHIX
      300      310
  
```

**Human**

SCORES Init1: 147 Initn: 147 Opt: 211  
 Smith-Waterman score: 211; 28.7% identity in 150 aa overlap

```

      110      120      130      140      150      160
MAELO      NLVPILARNGLFYAICDDGAWTQRLLELYYLNVLVKYWEADTVFLVLKKKPLEFLHYFH
      | : : : | : : : | : : : | : : : | : : : | : : : |
AC004050      SLLVVKDLTYLLPLCLPGDTIFILRKQKLIFFLEWHYH
      10      20      30

      170      180      190      200      210      220
MAELO      HSMIMVLCTVQVQGGYTSVSWVPITLNLTVHVFMYYYYMRSAGVRVWVK--QYLTTLQIV
      | : : : : : : : : : | : : | : : | : : | : : | : : | : :
AC004050      HITVLLYSWYSYKDMVAGGGWEMIMNYGVHVMYSYALRAAGFRVSRKFAMFTTSLQIT
      40      50      60      70      80      90

      230      240      250      260      270      280
MAELO      QFVLDLGFYIFCAYTYFAFTYFPWAPNVGKCGAGTEGAALFGCGLLSSYLLLFNFYRITY
      | : : : | : : : | : : : | : : : | : : : | : : : |
AC004050      QMLMG-----CVVNYLVFC---WMQH-DQCHSHFQNIWSSIMYLSYLVLFCHFFFEAY
      100      110      120      130      140
  
```

## Figure 17

SCORES Init1: 87 Initn: 218 Opt: 232  
 Smith-Waterman score: 272; 29.7% identity in 232 aa overlap

```

      40      50      60      70      80      90
MAELO  SFVFQEGVTPLSTQREVAMWTITYFVVIFGGRQIMKSQDAFKLKPLFILHNFLLTIASGS
      | : | : | | : : | : | : |
I05465  PRYKSQRMVPPGQLHPYVCLFCYLLTHCMAGTKIHEEPAAVLLPSILQLYNLGLTLLS--
      20      30      40      50      60      70

      100     110     120     130     140     150
MAELO  LLLLFIEENLVPIARNGLFYAICDDGAWTQRLELLYYL--NYLVKYWELADTVFLVLKKK
      | : | | : : : : | : : : : | | : | | : : | : : |
I05465  -LYMFYELVTGVWEGKYNFFCQGTRSAGESDMKIIIRVLWWYYFSKLIEFMDTFFFILRKN
      80      90     100     110     120

      160     170     180     190     200     210
MAELO  --PLEFLHYFHH-SMTMVLCFVQLGGYTSVSWVPITLNLTVHVFMYYYY-MRSAAGVR--
      : | | : | | : | : | : : | : | : | : | : | : | : |
I05465  NHQITVLHVYHHATMLNIWWFVMNWVPCGHSYFGATLNSFIHVLMYSSYGLSSIPSMRPY
      130     140     150     160     170     180

      220     230     240     250     260     270
MAELO  IWWKQYLTTLQIVQFVLDLGFYFCAYTYFAFTYFPWAPNVGKCAGTEGAALFGCGLLSS
      : | | : | : | : | | | : : | : : : | : | : | : |
I05465  LWWKYITQGQLVQFVLTIIQTTTCG-----VFWP-----CSFPLGWLFFQIGYMIS
      190     200     210     220     230

      280     290     300     310
MAELO  YLLLFINFYRITYNAKAKAAKERGSNFTPKTVKSGGSPKKPSKSKHIX
      : | | | | | | : : : :
I05465  LIALFTNFYIQTYNKKGASRRKEHLKGHQNGSVAAVNGHTNSFPSPLENSVKPRKQKDXQ
      240     250     260     270     280     290

```

**Figure 18**

1 MGTDQGKTFT WEELAAHNTK DDLLLAIRGR VYDVTKFLSR HPGGVDTL<sup>1</sup>LL  
51 GAGRDVTPVF EMYHAFGAAD AIMKKYYVGT LVSNELPIFP EPTVFHKT<sup>1</sup>IK  
101 TRVEGYFTDR NIDPKNRPEI WGRYALIFGS LIASY<sup>1</sup>YAQLF VPFVVERTWL  
151 QVVF<sup>1</sup>AIIMGF ACAQVGLNPL HDASHFSVTH NPTVWKILGA THDFFNGASY  
201 LVW<sup>1</sup>MYQHMLG HHPYTNIAGA DPDVSTSEPD VRR<sup>1</sup>IKPNQKW FVN<sup>1</sup>HNQHMF  
251 VPFLYGLLAF KVRIQDINIL YFVKTND<sup>1</sup>AIR VNPISTWHTV MFWGGKAFFV  
301 WYRLIVPLQY LPLGKVLLLF TVADMVSS<sup>1</sup>YW LALTFQANHV VEEVQWPLED  
351 ENGIIQK<sup>1</sup>DWA AMQVET<sup>1</sup>TQDY AHD<sup>1</sup>SHLWTSI TGSLNYQAVH HLF<sup>1</sup>PNVSQHH  
401 YPDILAI<sup>1</sup>IKN TCSEYKVPYL VKDTFWQ<sup>1</sup>AFA SHLEHLRVLG LRPKEE\*

## Response

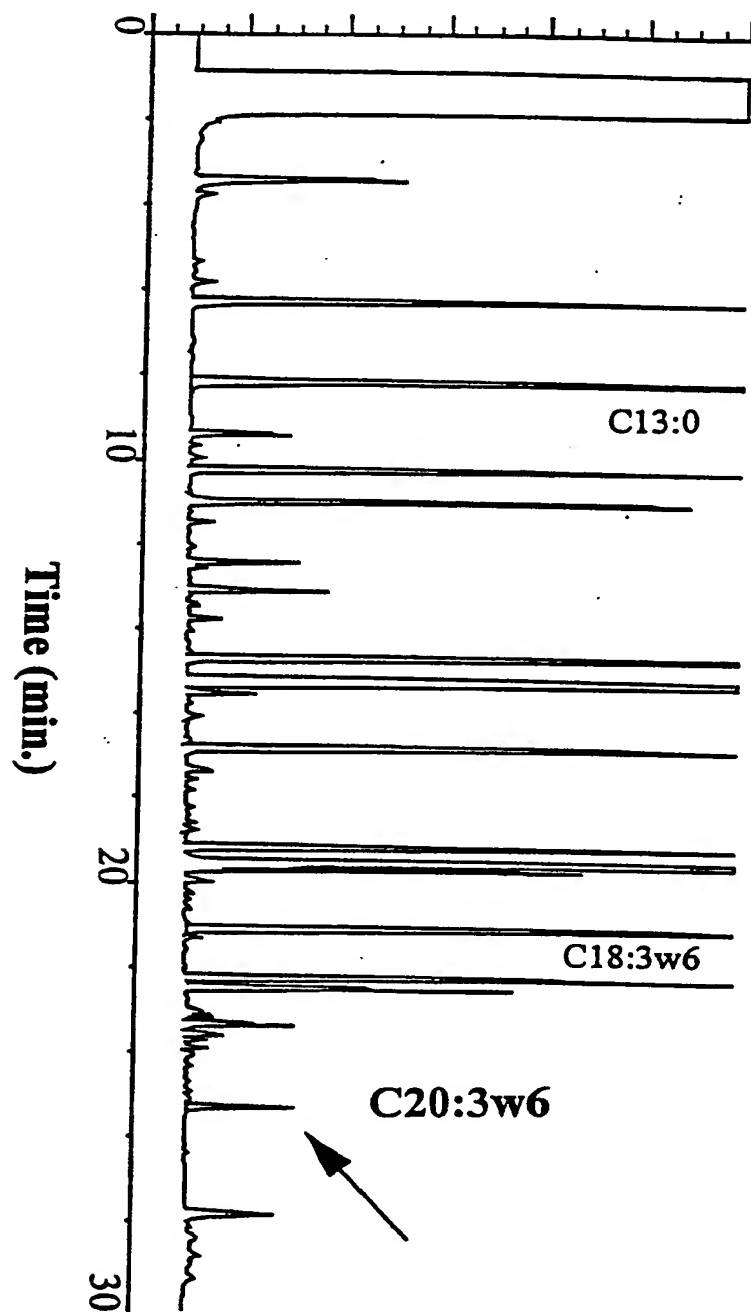
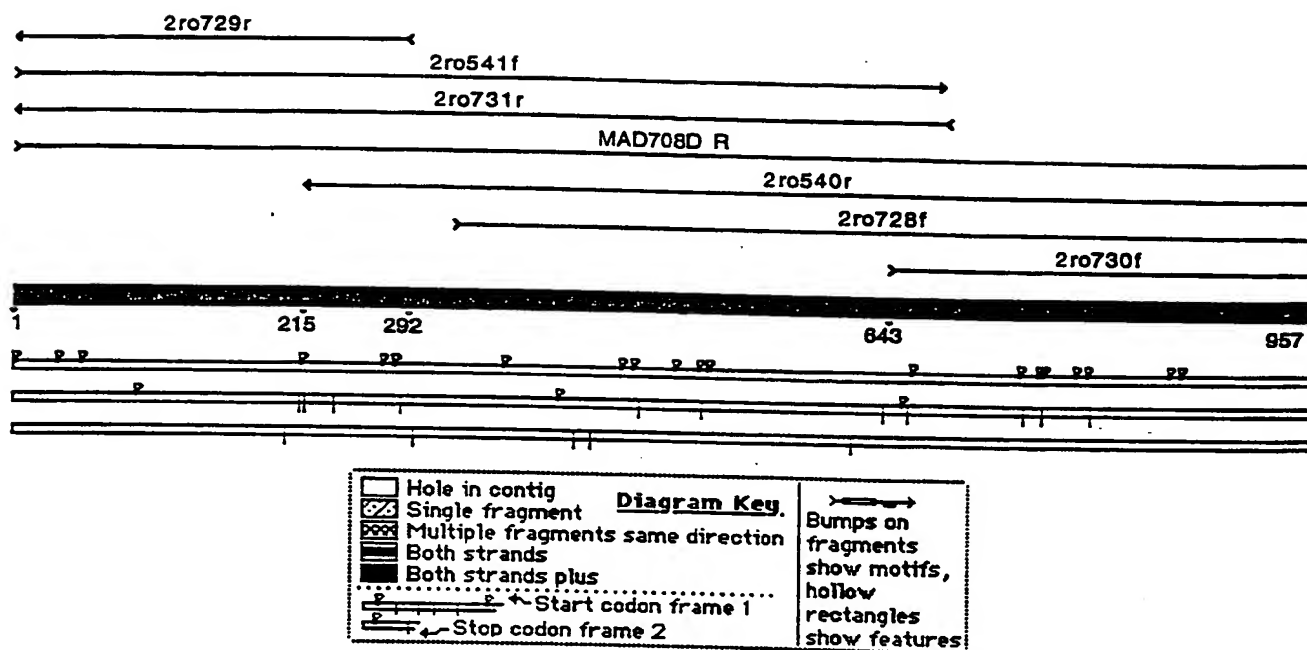


Figure 19





Figure 21



**Figure 22**

```
1  ATGGAGTCGA TTGCGCCATT CCTCCCATCA AAGATGCCGC AAGATCTGTT
51 TATGGACCTT GCCACCGCTA TCGGTGTCCG GGCCGCGCCC TATGTCGATC
101 CTCTCGAGGC CGCGCTGGTG GCCCAGGCCG AGAAGTACAT CCCCACGATT
151 GTCCATCACA CGCGTGGGTT CCTGGTCCGCG GTGGAGTCGC CTTTGGCCCG
201 TGAGCTGCCG TTGATGAACC CGTTCCACGT GCTGTTGATC GTGCTCGCTT
251 ATTTGGTCAC GGTCTTTGTG GGCATGCAGA TCATGAAGAA CTTTGAGCGG
301 TTCGAGGTCA AGACGTTTTT GCTCCTGCAC AACTTTTGTC TGGTCTCGAT
351 CAGCGCCTAC ATGTGCGGTG GGATCCTGTA CGAGGCTTAT CAGGCCAACT
401 ATGGACTGTT TGAGAACGCT GCTGATCATA CCTTCAAGGG TCTTCCTATG
451 GCCAAGATGA TCTGGCTCTT CTACTTCTCC AAGATCATGG AGTTTGTCGA
501 CACCATGATC ATGGTCCTCA AGAAGAACAA CCGCCAGATC TCCTTCTTGC
551 ACGTTTACCA CCACAGCTCC ATCTTCACCA TCTGGTGGTT GGTACACCTT
601 GTTGCACCCA ACGGTGAAGC CTACTTCTCT GCTGCGTTGA ACTCGTTCAT
651 CCATGTGATC ATGTACGGCT ACTACTTCTT GTCGGCCTTG GGCTTCAAGC
701 AGGTGTCGTT CATCAAGTTC TACATCACGC GCTCGCAGAT GACACAGTTC
751 TGCATGATGT CGGTCCAGTC TTCTGGGAC ATGTACGCCA TGAAGGTCCT
801 TGGCCGCCCC GGATACCCCT TCTTCATCAC GGCTCTGCTT TGGTTCTACA
851 TGTGGACCAT GCTCGGTCTC TTCTACAAC TTTACAGAAA GAACGCCAAG
901 TTGGCCAAGC AGGCCAAGGC CGACGCTGCC AAGGAGAAGG CAAGGAAGTT
951 GCAGTAA
```

**Figure 23**

1 MESIAPFLPS KMPQDLFMDL ATAIGVRAAP YVDPLEAALV AQAKEYIPTI  
51 VHHTRGFLVA VESPLARELP LMNPFHVLLI VLAYLVTVFV GMQIMKNFER  
101 FEVKTFSLLH NFCLVSISAY MCGGILYEAY QANYGLFENA ADHTFKGLPM  
151 AKMIWLFYFS KIMEFVDIMI MVLKKNRQI SFLHVVHHSS IFTIWWLVTF  
201 VAPNGEAYFS AALNSFIHVI MYGYYFLSAL GFKQVSFIKF YITRSQMTQF  
251 CMMSVQSSWD MYAMKVLGRP GYPFFITALL WFYMWTMLGL FYNFYRKNK  
301 LAKQAKADAA KEKARKLQ\*

Figure 24

Host(plasmid)	334(pRPB2)	334 (pYES2)
Added substrate	25µM GLA	25µM GLA
	(n=4)	
Fatty Acid	% total lipid	
C16:0	15.65	15.23
C16:1	35.2	38.59
C18:0	5.68	5.55
C18:1n-9	25.55	25.27
C18:3n-6	3.1	6.75
C20:0	0.36	0.14
C20:3n-6	(62.0%) 5.06	(2.6%) 0.18
Total Lipid (µg)	314	247
(% conversion) = product/(substrate+product)		

Figure 25

A.									
Host(plasmid)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)
Added substrate	25µM SA	25µM OA	25µM LA	25µM DGLA	25µM AA	25µM Adrenic	25µM AA	25µM AA	25µM Adrenic
	C18:0	C18:1n-9	C18:2n-6	C20:3n-6	C20:4n-6	C22:4n-6			
Fatty Acid	% total lipid								
C16:0	15.07	14.52	15.74	15.69	16.06	15.15			
C16:1	33.7	32.37	32.23	25.65	33.65	33.39			
C18:0	*9.78	5.83	5.61	8.33	4.52	5.35			
C18:1n-9		*37.25	26.05	20.15	24.54	28.54			
C18:2n-6			*10.4						
C18:3n-6									
C20:2n-6			0.29						
C20:3n-6				*16.5					
C20:4n-6				0.27	*11.7				
C22:4n-6						*7.46			
Total Lipid (µg)	132	130	171	55	225	163			
B.									
Host(plasmid)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)
Added substrate	25µM ALA	25µM STA	25µM EPA	25µM EPA	25µM EPA	25µM EPA	25µM EPA	25µM EPA	25µM EPA
	C18:3n-3	C18:4n-3	C20:5n-3	C20:5n-3	C20:5n-3	C20:5n-3	C20:5n-3	C20:5n-3	C20:5n-3
Fatty Acid	% total lipid								
C16:0	17.32	16.01	20.67	20.67	20.67	20.67	20.67	20.67	20.67
C16:1	27.68	34.31	50.7	50.7	50.7	50.7	50.7	50.7	50.7
C18:0	6.75	5.39	6.14	6.14	6.14	6.14	6.14	6.14	6.14
C18:1n-9	28.4	28.54							
C18:3n-3	*8.39								
C18:4n-3		*1.95							
C20:4n-3		(73.2%) 5.33							
C20:5n-3			*10.33						
C22:5n-3			0.25						
Total Lipid (µg)	114	199	201	201	201	201	201	201	201

\*Indicates substrate added  
 (% conversion) = product/(substrate+product)

Figure 26

A.

Host(plasmid)	334(pRPB2+PRPE31)	334(pYES2+pYX242)
Added substrate	25µM GLA	25µM GLA
Fatty Acid	% total lipid	
C16:0	15.54	18.26
C16:1	30.16	33.51
C18:0	8.76	5.58
C18:1n-9	27	27.37
C18:3n-6	*2.6	*5.6
C20:0	0.4	0.32
C20:3n-6	(57.4%) 3.55	(2.9%) 0.17
C20:4n-6	(27.6%) 1.32	ND
Total Lipid (µg)	254	258

B.

Host(plasmid)	334(pRPB2+PRPE31)	334(pYES2+pYX242)
Added substrate	25µM STA	25µM STA
Fatty Acid	% total lipid	
C16:0	18	16.4
C16:1	28.37	34.78
C18:0	7.42	5.71
C18:1n-9	26.44	30.15
C18:4n-3	*2.93	*4.57
C20:0	0.25	0.17
C20:4n-3	4.13	0.32
C20:5n-3	(39%) 1.87	(2.1%) .10
Total Lipid (µg)	257	304

\* indicates substrate added  
 (% conversion) = product/(substrate+product)

28/51

## Figure 27

SCORES Init1: 114 Initn: 278 Opt: 278  
 Smith-Waterman score: 308; 30.9% identity in 259 aa overlap

	40	50	60	70	80	90	99
GLELO	VAQAERYIPTIVHETRGFLVAVESPLARELPLMNPFFHVLLIVLAYLVTVFVGMQIMKNFE						
	: :   : : : : : : :       : :						
MAELO	GIKLDITYFAQAYELVTGKSIDSFVFQEGVTPLSTQREVAMWTITYFVVIFGGRQIMKSQD						
	20	30	40	50	60	70	
	100	110	120	130	140	150	
GLELO	RPEVKTFSLLHNFCLVSISAYMCGGILYE--AYQANYGLFENAADHTFKGLPMARKMIWLF						
	: : : :   : : : : : : :       : : : :						
MAELO	AFKLEPLFLLHNFLLTIASGSLLLLFIENLVPILARNGLFYAICDDGAWTORLELLYYLN						
	80	90	100	110	120	130	
	160	170	180	190	200	210	
GLELO	YFSKIMEFVDTMIMVLKKNRQISFLHVIHSSIFTIWWLVTFVAPNGEAYFSAALNSFI						
	:   :						
MAELO	YLVKYWELADTVFLVLKK--KPLEFLHYFHHS--MTMVLCFVQLGGYTSVSWVPITLNLTV						
	140	150	160	170	180	190	
	220	230	240	250		260	
GLELO	HVIMYGYFFLSALGFKQVSFIKFIYITRSQMTQF-----				CMMSVQS-----		SWDMYAM
	:     :   : : :   :   : :				: : :		:
MAELO	HVFMYYYMRSAAAGVRI--WWKQYLTTLQIVQFVLDLGFIFYFCAITYFAITYFPWAPNVG						
	200	210	220	230	240	250	
	270	280	290	300	310		
GLELO	KVLGRPGYPFFITALLWFYMWMTMLGLFYNFYRKNAKLAKQAKADAAKEKARKLQ						
	:   :   :         : : :       : :						
MAELO	KCAGTEGAALFGCGLLSSYLL----LFINFYR----ITYNAKAKAAKERGSNFTPKTVKS						
	260	270	280	290	300		
MAELO	GGSPKKPKSKKHIX						
	310						

29/51

Figure 28

GLELO	1	-HES	42
MAELO	1	IA	20
GNS1	1	PF	36
SUR4	1	LP	42
GLELO	43	AEK	85
MAELO	21	LD	63
GNS1	37	EH	79
SUR4	43	W	84
GLELO	96	VT	128
MAELO	64	IV	104
GNS1	80	FG	120
SUR4	85	IS	127
GLELO	129	-A	167
MAELO	105	Y	147
GNS1	121	EN	163
SUR4	128	IG	170
GLELO	168	IM	210
MAELO	148	ME	187
GNS1	164	FA	203
SUR4	171	RR	210
GLELO	211	AA	253
MAELO	186	ST	228
GNS1	204	SL	244
SUR4	211	EN	251
GLELO	254	SM	296
MAELO	229	OG	270
GNS1	245	VE	286
SUR4	252	VE	294
GLELO	297	KN	318
MAELO	271	AK	313
GNS1	287	KA	329
SUR4	295	AK	337
MAELO	314	SR	317
GNS1	330	KN	347
SUR4	338	TR	345



30/51

## Figure 29

SCORES Initl: 83 Initn: 186 Opt: 271  
 Smith-Waterman score: 297; 28.5% identity in 242 aa overlap

	30	40	50	60	70	80
MAELO	YELVTGKSIDSFVFQEGVTPPLSTQREVAMWTITYFVVIFGGRQIMKSQDAFKLKPLFILH					
					::   -:::   :  :::   : : :::	
HS1	STYFKALLGPRDTRVKGWFLLDNYIPTFICSVIYLLIVWLGPKYMRNKQPFSCRGILVVY					
	10	20	30	40	50	60
	90	100	110	120	130	140
MAELO	NELLTIASGSLLLFIENLVPILARNGLFYAICDDGAWTQRLLELLYYL--NYLVKYWELA					
	:   :   :   :   : : : :   :   : : :   :   :   :					
HS1	NLGLTLLS---LYMFCELVGTGVWEGKYNFFCQGTRTAGESDMKIIRVLWWYYFSKLIEFM					
	70	80	90	100	110	120
	150	160	170	180	190	200
MAELO	DTVFLVLKK--KPLEFLHYFHH-SMTMVLCFVQLGGYTSVSWVPITLNLTVHVFMYYYY-					
	: :   : :   :   :   :   :   :   :   :   :   :   :   :   :					
HS1	DTFFFILRKNNHQITVLHVYHHASMLNIWWFVMNWVPCGHSYFGATLNSFIHVLMSYYG					
	130	140	150	160	170	180
	210	220	230	240	250	260
MAELO	MRSAGVR--IWWKQYLTTLQIVQFVLDLGFIFYFCAYTYFAFTYFPWAPNVGKAGTEGA					
	:   : :   :   :   :   :   :   :   :   :   :   :   :   :   :   :					
HS1	LSSVPSMRPYLWKKYITQGQLLQFVLTII-IQTSCGVI-----W-P-----CTFPLGW					
	190	200	210	220		230
	270	280	290	300	310	
MAELO	ALFGCGLLSSYLLLFINFYRITYNAKAKAAKERGSNFTPKTVKSGGSPKKPSKSKHI					
	:   :             : : : :					
HS1	LYFQIGYMISLIAFTNFYIQTYNKKGASRRKDHLKDHQNGSMAAVNGHTNSFSPLENNV					
	240	250	260	270	280	290
HS1	KPRKLRKDX					
	300					

31/51

Figure 30

SCORES Initl: 88 Initn: 208 Opt: 272

Smith-Waterman score: 279; 28.2% identity in 266 aa overlap

```

      30      40      50      60      70      80
MAELO  QAYELVTGKSIDSFVFQEGVTPLESTQREVAMWTTITYFVVFVGGROIMKSQDAFKLKPLFI
      : | : ||| : : | | | : : | : | : : |
HS2    VNLYQEVMMKHADPRIQGYPLMGSPILLMTSILLTYVYFVLSLGPRI-MANRKPFFQLRGFMI
      10      20      30      40      50      60

      90      100     110     120     130     140
MAELO  LHNFLLTIASGSLILLFTIEN--LVPILAR-NGLFYAICDDGAWTORLELLYYLNYLVKYW
      : : || | : | : : | : : | : : | : | : : : | :
HS2    VYNFSLVALSLYIVYEFLMSGWLSTYTWRCDFVDYSNSPEALRMVRVAVLFLFS---KFI
      70      80      90      100     110     120

      150     160     170     180     190
MAELO  ELADIVFVLVKKK--PLEFLHYFHHSMT----MVLCFVQLGGYTSVSWVPITLNLTVHVF
      || ||| : : : | : ||| ||| : : || : | : : | : ||| :
HS2    ELMDTVIFILRKKGQVTFLLHVFHHSVLPWSWWWGVKIAPGGMGFSHAM---INSSVHVI
      130     140     150     160     170

      200     210     220     230     240
MAELO  MYYYMRSAGV----RIWWKQYLTLQIVQFVL---DLGFIYF---CAYTYFAFTYFPW
      || || | | | : ||| : : : : : ||| : : || | | | : : : |
HS2    MYLYYGLSAFGPVAQPYLWKKHMTAIQLIQFVLVSLHISQYYFMSSCNQYQPVIIHLIW
      180     190     200     210     220     230

      250     260     270     280     290     300
MAELO  APNVGKCACTEGAALFGCGLLSYLLLFINFYRITYNAKAKAAKERGSNFTPKTVKSGGS
      : : | : : : || || : : | : : : | : | : |
HS2    -----MYG---TIFFMLFSNFWYHSYTKGKRLPRALQONGAPGIAKVKAN
      240     250     260     270

      310
MAELO  PKKPSKSKHI
HS2    X
      280

```

32/51

## Figure 31

SCORES Init1: 88 Initn: 207 Opt: 223  
Smith-Waterman score: 236; 30.4% identity in 191 aa overlap

```

      100      110      120      130      140      150
MAELO  LLLLFTEINLVPILARNGLFYAICDDGAWIQRLLELLYYLNVLVKYWELADTVFLVLKKKP-
      | : : | : | | | | | : : | |
MM2    IVYEFILMSGWLSTYTWRCDPIDFSNSPEALRMVRVAVLWFLSKVIELMDTVIFILRKKDG
      20      30      40      50      60      70

      160      170      180      190      200      209
MAELO  -LEFLHYFHHSMTMVLCF----VOLGGYTSVSWVPITLNLTVHVFMYYYMRSAGV---
      : | | | | | : : : | : | : | | | | | | | | | |
MM2    QVTFLHVFHHSVLPWSWWWGIKIAPGGMGSFHAM---INSSVHVVMYLYYGLSALGPVAQ
      80      90      100      110      120      130

      210      220      230      240      250      260
MAELO  -RIWWKQYLTTLQIVQFVL---DLGFIYF---CAYTYFAFTYFPWAPNVGKAGTEGAAL
      : | | | : : : | : | | | : : | | | : : : |
MM2    PYLWWKKHMTAIQLIQFVLVSLHISQYYFMPSCNYQYPVTHHLIW-----M
      140      150      160      170

      270      280      290      300      310
MAELO  FGCGLLSSYLLLFINFYRITYNAKAKAAKERGSNFTPKTVKSGGSPKKPSKSKI
      : | : : : | | : : : : : : : | : | : |
MM2    YG----TIFFILFSNFWYHSYTKGKRLPRAVQONGAPATTKVKAN
      180      190      200      210
```

## Figure 32

SCORES Initl: 51 Initn: 115 Opt: 168  
Smith-Waterman score: 168; 30.4% identity in 115 aa overlap

MAELO	30	40	50	60	70	80
	YELVTGKSIDSFVFQEGVTPLSTQREVAMWTITYFVWIFGGRQIMKSQDAFKLKPLFILH					
AI225632	NAFLDNMFGRDSDRVRGWFLDLSYLPFTILTITVLLSIWLGKYMKNRPALSLRGILTLY					
	20	30	40	50	60	70
MAELO	90	100	110	120	130	140
	NELLTIASGSLLLLFIEINLVPILARNGLFYAICDD---GAWTORLELLYYLNYLVKYWE					
AI225632	NLAITLLSAYMLVELI-----LSSWEGGYNLQCNLDSAGEGDVRVAKVLVWYYF SKLVE					
	80	90	100	110	120	
MAELO	150	160	170	180	190	200
	LADTVFLVLKKK--PLEFLHYFHSMIMVLCFVQLGGYTSVSWVPITLNLTVHVFMYYYY					
AI225632	FLDTIFFVLRKKANQITFLHVVYHHASMFNI					
	130	140	150			



Figure 34

SCORES Init1: 316 Initn: 384 Opt: 477  
 Smith-Waterman score: 477; 34.2% identity in 240 aa overlap

	50	60	70	80	90	100
GLELO	AQAKEYIPTIVHHTRGFLVAVESPLARELPLMNPFFHVLLIVLAYLVTVFVGMQIMKNFER					
HS1	MEHFDASLSTYFKALLGPRDTRVKGWFLLDNYIPTFICSVIYLLIVWLGPKYMRNKQP					
	10	20	30	40	50	
	110	120	130	140	150	159
GLELO	FEVKTFSLHNFCLVSISAYMCGGILYEAYQANYGLF-ENAAHDTFKGLPMAKMIWLFYF					
HS1	FSCRGILVVYNLGLTLLSLYMFCELVGTGVWEGKYNFFCQGTRTAGESDMKIIRVLWWYYF					
	60	70	80	90	100	110
	160	170	180	190	200	210
GLELO	SKIMEFVDTMIMVLKKNRQISFLHVYHSSIFTIWWLVTFVAPNGEAYFSAALNSFIHV					
HS1	SKLIEFMDTFFFILRKNNHQITVLHVYHHSMLNIWWFVMNWWPCGHSYFGATLNSFIHV					
	120	130	140	150	160	170
	220	230	240	250	260	270
GLELO	IMYGYFSLSAL-GFKQVSFIKFYITRSQMTQFCMMSVQSSWDMYAMKVLGRPGYPFFITA					
HS1	LMYSYYGLSSVPSMRPYLWWKKYITQGQLLQFVLTIIQTS-----CGVIWPCTFPLGWLY					
	180	190	200	210	220	230
	280	290	300	310		
GLELO	LLWFYMWMTMLGLFYNFYRK--NAKLAKQAKADAKEKARKLQ					
HS1	FQIGYMISLIALFTNFYIQTYNKKGASRRKDHLKDHQNGSMAAVNGHTNSFSPLENNVKP					
	240	250	260	270	280	290

## Figure 35

SCORES Init1: 80 Initn: 114 Opt: 178  
Smith-Waterman score: 178; 28.8% identity in 146 aa overlap

	140	150	160	170	180	190
GLELO	FENAADHTFKGLPMAKMIWLFYFSKIMEFVDTMIMVLKNNRQISFLHVYHHSSIFTIWW					
AC004050	DTIFIILRK--QKLIFLHWYHHITVLLYSW					
				10	20	
	200	210	220	230	240	250
GLELO	LVTfVAPNGEAYFSAALNSFIHVIMYGYFLSALGFKQVSFIKFIYITRSQMTQFCMMSVQ					
AC004050	YSYKDMVAGGGWF-MTMNYGVHAVMYSYYALRAAGFRVSRKFAMFITLSQITQMLMGCVV					
	30	40	50	60	70	80
	260	270	280	290	300	310
GLELO	SSWDMYAMKVLGRPGYPFFITALLW--FYMWTMLGLFYNFYRKN--AKLAKQAKADAAKE					
AC004050	NYLVFCWMQ--HDQCHSHF-QNIFWSSLMYLSYLVLFCHFFFEAYIGKMRKTTKAEX					
	90	100	110	120	130	140
GLELO	KARKLQ					

37/51

## Figure 36

SCORES Init1: 288 Initn: 288 Opt: 399  
 Smith-Waterman score: 399; 34.6% identity in 211 aa overlap

	80	90	100	110	120	130
GLELO	LLIVLAXLVTVFVGMQIMKNFERFEVKTFSLLHNFCLVSISAYMCGGILYEAYQANYGLF					
	:::      :     : : :					
MM2	IVYNFSLVILSLYIVYEFILMSGWLSTYTW					
			10		20	30
	140	150	160	170	180	190
GLELO	ENAAD--HTFKGLPMAKMIWLFYFSKIMEFVDTMIMVLKKNNRQISFLHVVYHSSIFTIW					
	:   : : :   : : :   : : :   : : :   : : :   : : :   : : :					
MM2	CDPIDFSNSPEALRMVRVAWLFMLSKVIELMDTVIFILRKKDQVTFHLHVFHHSVLPWSW					
	40	50	60	70	80	90
	200	210	220	230	240	250
GLELO	WLVTVPAPNGEAYFSAALNSFIHVIMYGYFLSALGFKQVSFI--KFYITRSQMTQFCMM					
	: : :   : : :   : : :   : : :   : : :   : : :   : : :					
MM2	WWGIKIAPGGMGSFHAMINSSVHVVMYLYYGLSALGPVAQPYLWWKKHMTAIQLIQFVLV					
	100	110	120	130	140	150
	260	270	280	290	300	309
GLELO	SVQSSWDMYAMKVLGRPGYPFFITALLWFYMWMTMLGLFYNF----YRKNAKLAKQAKADA					
	: :   : :   : :   : :   : :   : :   : :   : :   : :   : :					
MM2	SLHIS-QYYFMPSCNYQ-YPVLIH-LIWMYGTIFFILFSNEFWYHSYTKGKRLPRAVQONG					
	160	170	180	190	200	
	310					
GLELO	AKEKARKLQ					
MM2	APATTKVKAN					
	210					



## Figure 37

SCORES Init1: 160 Initn: 227 Opt: 269  
Smith-Waterman score: 269; 35.3% identity in 119 aa overlap

	50	60	70	80	90	100
GLELO	PTIVHHTRGFLVAVESPLARELPLMNPFFHVLLIVLAYLVTVFVGMQIMKNFERFEVKTFS					
AI225632	NEVNAFLDNMFGPRDSRVRGWFLDSYLPFTILTITYLLSIWLGKNKYMKNRPALSLRGIL					
	10	20	30	40	50	60
	110	120	130	140	150	160
GLELO	LLHNFCLVSISAYMCGGILYEAYQANYGLFENAADHTFKG-LPMAK-MIWLIFYFSKIMEF					
AI225632	TLYNLAITLLSAYMLVELILSSWEGGYNLQCNLDSAGEGDVRVAKVLVW-YYFSKLVEF					
	70	80	90	100	110	120
	170	180	190	200	210	220
GLELO	VDTMIMVLKKNNRQISFLHVYHHSSIFTIWWLVTFVAPNGEAYFSAALNSFIHVIMYGY					
AI225632	LDTIFFVLRRKANQITFLHVYHHASMFNI					
	130	140	150			

39/51

## Figure 38

SCORES Init1: 64 Initn: 129 Opt: 233  
 Smith-Waterman score: 239; 23.7% identity in 279 aa overlap

	20	30	40	50	60	70
GLELO	FMDLATAIGVRAAPYVDPLEAALVAQAKEYIPTIVHHTRGFLVAVESPLAREL-----PL					
U97107	MDTSMNFSRGLKMDLMQPYDFETFDLRF					
				10	20	30
	80	90	100	110	120	129
GLELO	MNPFFHV--LLIVLAYLVTFVGMQIMKNFERFEVKTFSLHNFCLVSIAYMCGGILYEA					
U97107	LEEWVSSFLIVVVYLLLVVGQTYMTRKSFSLQRPILWSFFLAIFS--ILGTLRMWK					
	40	50	60	70	80	
	130	140	150	160	170	180
GLELO	YQAN----YGLFENAADHTFKGLPMAMIW--LFYFSKIMEFVDTMIMVLKKNRQISFL					
U97107	FMATVMFTVGLKQTVCFAYTDDAVVRF-WSFLFLLSKVVELGDTAFIILRK--RPLIFV					
	90	100	110	120	130	140
	190	200	210	220	230	240
GLELO	HVVYHSSI--FTIWWLVTFVAPNGEAYFSAALNSFIHVIMYGYFFLSALGFKQVSFIKFY					
U97107	HWYHHSTVLLFTSFGYKNKV-PSGGWFMT--MNFVHVSVMYTYTMTKAAKLKHPNLLPMV					
	150	160	170	180	190	200
	250	260	270	280	290	
GLELO	ITRSQMTQFCMMSVQSSWDMYAMKVLG--RPGYPFFITALLWFYMWMTMLGLFYN--FYRK					
U97107	ITSLQILQMVLTIFGILNYIWRQEKGCCHTTTEHFFWSFMYLGYTYFILFAHFFHRAYLRLP					
	210	220	230	240	250	260
	300	310				
GLELO	NAKLAKQAKADAAKEKARKLQ					
U97107	KGKVASKSQ					
	270					

## Figure 39

SCORES Init1: 100 Initn: 205 Opt: 271  
 Smith-Waterman score: 271; 30.7% identity in 218 aa overlap

	60	70	80	90	100	110
GLELO	TRGFLVAVESPLARELPLMNPFHVLLIVLAYLVTVFVGMQIMKNFERFEVKTFSLLNFC					
				: :	: :  : :	:
U68749	ATHGPKNFPDAEGRKFFADHFDVTIQASILYMVVVFGTKWFMNRNRPQFQLTIPLNINWFI					
(F56H11.4)	30	40	50	60	70	80
	120	130	140	150	160	
GLELO	LVSISAYMCGGILYEAYQ--ANYGL---FENAADHTFKGLPMAMIWLFYFSKIMEFVDT					
	::	: :	:	:::		: : : : : :
U68749	LAAFSTIAGAVKMTPEFFGTIANKGIVASYCKVFDEFT-KG-ENGYVWVWLFMASKLFELVDT					
(F56H11.4)	90	100	110	120	130	140
	170	180	190	200	210	220
GLELO	MIMVLKKNNRQISFLHVVYHHSSIFTIWWLVTFVAPNGEAYFSAALNSFIHVIMYGYFSL					
	::: :	:		:	:	: : : : :
U68749	IFLVLRK--RPLMFLHWYHHILTMIYAWYSHPLTP-GFNRYGIYLNFEVHAFMYSYFLR					
(F56H11.4)	150	160	170	180	190	
	230	240	250	260	270	280
GLELO	ALGFKQVSFIKFYITRSQMTQFCMMSVQSSWDMYAMKVLGRP-GYPFFITALLWFYMWTM					
	:::	:		: :	:	: : : : :
U68749	SMKIRVPGFIAQAITSLOIVQFIISCAVLAHLGYLMHFTNANCDPEPSVFKLAVFMDTTY					
(F56H11.4)	200	210	220	230	240	250
	290	300	310			
GLELO	LGLFYNFYRKNAKLAKQAKADAKEKARKLQ					
	:	:				
U68749	LALFVNFFLQSYVLRGGKDKYKAVPKKKNN					
(F56H11.4)	260	270	280			

	10	20	30	40	50	59
MAELO	MAAAILDKVNFGIDQPFGIKLDTYFAQAYELVTGKSIDSFVFQEGVTPLPSTQREVAMW-T					
	:	:	:	:	:	:
U68749	MAQHPLVQRLLDVKFDT---KRFVAIATHGPKNFPDAEGRKFFADHFDVTIQAS					
(F56H11.4)	10	20	30	40	50	
	60	70	80	90	100	110
MAELO	ITYFVVIFGGRIIMKSQDAFKLK-PLFILHNFLLTIASGSLLLLFIENLVPILARNGLFY					
		:	:	:	:	:
U68749	ILYMVVVFGTKWFMRNRQPFLTIPLNIW-NFILAAFSIAGAVKMTPEFFGTIANKGIVA					
(F56H11.4)	60	70	80	90	100	110
	120	130	140	150	160	170
MAELO	AICDDGAWTQRLELLYYLNYLV-KYWELADTVFLVLKKKPLEFLHYFHHSMTMVLCFVQL					
	:	:	:	:	:	:
U68749	SYCKVFDFTKGENGYVWVLFMASKLFELVDITFLVLRKRPLMFHLHWYHHILTMIYAWYSH					
(F56H11.4)	120	130	140	150	160	170
	180	190	200	210	220	230
MAELO	GGYTSVSWVPITLNLTVHVFMY-YYYMRSAAGVRI--WWKQYLTTLQIVQFVLDLGFIFY					
	:	:	:	:	:	:
U68749	PLTPGFNRYGIYLNFNHFVVFHAFMYSYYFLRSMK-IRVPGFIAQAITSLQIVQFIISCAVLAH					
(F56H11.4)	180	190	200	210	220	
	240	250	260	270	280	
MAELO	CAYT-YFAFTYFPWAPNVGKCAGTEGAALFGCGLLSSYLLLFINFYRITY-----NAKAK					
	:	:	:	:	:	:
U68749	LGYLMHFTNANCDFEPSVFKLAE----VF---MDTTYLALFVNFELQSYVLRGGKDQKYK					
(F56H11.4)	230	240	250	260	270	280
	290	300	310			
MAELO	AAKERGSNFTPPTVKSGGSPKKPSKSKHI					
	:	:	:			
U68749	AVPKKKNN					
(F56H11.4)						

42/51

## Figure 41

SCORES Init1: 77 Initn: 155 Opt: 264  
Smith-Waterman score: 264; 27.2% identity in 206 aa overlap

	40	50	60	70	80	90
GLELO	AALVAQAEKYIPTIVHHTRGFLVAVESPLARELPLMNPFFHVLLIVLAYLVTVFVGMQIMK					
DM1	PTKMINMDISVTPNYSYIFDFENDFIHQTRKWMLNWTWVFYFCGIYMLVIFGGQHFMQ					
	10	20	30	40	50	60
	100	110	120	130	140	150
GLELO	NFERFEVKTFSLLHNFCIVSISAYMCGGILYEAYQA--NYGLFENAADHTF--KGLPMAK					
DM1	NRPRFQLRGPLIIWNTLLAMFSIMGAARTAPELIHVLRHYGLFHSVCVPSYIEQDRVCGF					
	70	80	90	100	110	120
	160	170	180	190	200	210
GLELO	MIWLFYFSKIMEFVDTMIMVLKKNRQISFLHVYHHSSIFTIWWLVTFVAPNGEAYFSAA					
DM1	WTWLFVLSKLPGLGDTIFIVLRK--QPLIFLHWYHHITVLIYSWF-SYTEYTSSARWFIV					
	130	140	150	160	170	180
	220	230	240	250	260	270
GLELO	LNSFIHVIMYGYFSLGFKQVSFIKFYITRSQMTQFCMMSVQSSWDMYAMKVLGRPGY					
DM1	MNYCVHSMYSYALKAAARNPPRFISMIITSLQLAQMIIGCAINWANGFLKTHGTXSC					
	190	200	210	220	230	240
	280	290	300	310		
GLELO	PFFITALLWFYMWMTMLGLFYNFYRKNKAKLAKQAKADAAKEKARKLQ					
DM1	HISQRNINLSIAMYSSYFVLFARFFYKAYLAPGGHKSRRMA					
	250	260	270	280		

[illegible]

## Figure 43

```
1  ATGGAACATT TTGATGCATC ACTTAGTACC TATTTCAAGG CATTGCTAGG
51 CCCTCGAGAT ACTAGAGTAA AAGGATGGTT TCTTCTGGAC AATTATATAC
101 CCACATTTAT CTGCTCTGTC ATATATTTAC TAATTGTATG GCTGGGACCA
151 AAATACATGA GGAATAAACA GCCATTCTCT TGCCGGGGGA TTTTAGTGGT
201 GTATAACCTT GGA CTCACAC TGCTGTCTCT GTATATGTTT TGTGAGTTAG
251 TAACAGGAGT ATGGGAAGGC AAATACAAC TCTTCTGTCA GGGCACACGC
301 ACCGCAGGAG AATCAGATAT GAAGATTATC CGTGTCTCTT GGTGGTACTA
351 CTTCTCCAAA CTCATAGAAT TTATGGACAC TTTCTTCTTC ATCCTGCGCA
401 AGAACAACCA CCAGATCACG GTCCTGCACG TCTACCACCA TGCCTCGATG
451 CTGAACATCT GGTGGTTTGT GATGAAC TGGTGGTGG GTCCCCTGCG GCCACTCTTA
501 TTTTGGTGCC AACTTAATA GCTTCATCCA CGTCCCTCATG TACTCTTACT
551 ATGGTTTGTC GTCAGTCCCT TCCATGCGTC CATACTCTG GTGGAAGAAG
601 TACATCACTC AGGGGCAGCT GCTTCAGTTT GTGCTGACAA TCATCCAGAC
651 CAGCTGCGGG GTCATCTGGC CGTGCACATT CCCTCTTGGT TGGTTGTATT
701 TCCAGATTGG ATACATGATT TCCCTGATTG CTCTCTTCAC AAACCTTCTAC
751 ATTCAGACCT ACAACAAGAA AGGGGCCTCC CGAAGGAAAG ACCACCTGAA
801 GGACCACCAG AATGGGTCCA TGGCTGCTGT GAATGGACAC ACCAACAGCT
851 TTTCACCCCT GGAAAACAAT GTGAAGCCAA GGAAGCTGCG GAAGGATTGA
901 AGTCAAAGAA TTGA
```

## Figure 44

1 MEHFDASLST YFKALLGPRD TRVKGWFLLD NYIPTFICSV IYLLIVWLGP  
51 KYMRNKQPFS CRGILVVYNL GLTLLSLYMF CELVTGVWEG KYNFFCQGTR  
101 TAGESDMKII RVLWWYYFSK LIEFMDTFFF ILRKNNHQIT VLVVYHHASM  
151 LNIWWFVMNW VPCGHSYFGA TLNSFIHVLN YSYYGLSSVP SMRPYLWWKK  
201 YITQGQLLQF VLTIIQTSCG VIWPCTFPLG WLYFQIGYMI SLIALFTNFI  
251 IQTYNKKGAS RPKDHLKDHI NGSMAAVNGH TNSFSPLENN VKPRKLRKD\*



Figure 45

Host (plasmid)	334(pYX242)	334(pRAE-58-A1)	334(pYX242)	334(pRAE-58-A1)
Added substrate	25 $\mu$ M GLA	25 $\mu$ M GLA	25 $\mu$ M AA	25 $\mu$ M AA
Fatty acid	%total fatty acid	%total fatty acid	%total fatty acid	%total fatty acid
C18:3n-6	4.40	2.71	0.03	0.04
C20:3n-6	0.09	(50.34%)* 2.75	0.02	0.02
C20:4n-6			7.84	3.97
C22:4n-6			ND	(23.37%)* 1.21
C16:1n-7	41.11	34.72	41.49	35.07
C18:1n-7	1.85	11.33	2.01	11.57
C20:1n-7	0.04	1.48	0.04	1.62
C18:1n-9	15.60	15.66	15.16	14.57
C20:1n-9	0.06	0.22	0.06	0.23
C18:1n-5	0.11	0.62	0.12	0.58
Total Lipid	370	969	359	514
*% conversion=product/(substrate+product)				

## Figure 46

```
1  ATGGCTCAGC ATCCGCTCGT TCAACGGCTT CTCGATGTCA AATTCGACAC
51  GAAACGATTT GTGGCTATTG CTACTCATGG GCCAAAGAAT TTCCCTGACG
101 CAGAAGGTCG CAAGTTCTTT GCTGATCACT TTGATGTTAC TATTCAGGCT
151 TCAATCCTGT ACATGGTCGT TGTGTTCCGA ACAAATGGT TCATGCGTAA
201 TCGTCAACCA TTCCAATTGA CTATTCCACT CAACATCTGG AATTTTCATCC
251 TCGCCGCATT TTCCATCGCA GGAGCTGTCA AAATGACCCC AGAGTTCTTT
301 GGAACCATTG CCAACAAAGG AATTGTCGCA TCCTACTGCA AAGTGTTTGA
351 TTTCACGAAA GGAGAGAATG GATACTGGGT GTGGCTCTTC ATGGCTTCCA
401 AACTTTTCGA ACTTGTTGAC ACCATCTTCT TGGTTCTCCG TAAACGTCCA
451 CTCATGTTCC TTCCTGGTA TCACCATATT CTCACCATGA TCTACGCCTG
501 GTACTCTCAT CCATTGACCC CAGGATTCAA CAGATACGGA ATTTATCTTA
551 ACTTTGTCGT CCACGCCTTC ATGTACTCTT ACTACTTCCT TCGCTCGATG
601 AAGATTTCGCG TGCCAGGATT CATCGCCCAA GCTATCACAT CTCTTCAAAT
651 CGTTCAATTC ATCATCTCTT GCGCCGTTCT TGCTCATCTT GGTTATCTCA
701 TGCATTTCAC CAATGCCAAC TGTGATTTTC AGCCATCAGT ATTCAAGCTC
751 GCAGTTTTCA TGGACACAAC ATACTTGGCT CTTTTCGTCA ACTTCTTCCT
801 CCAATCATAT GTTCTCCGCG GAGGAAAAGA CAAGTACAAG GCAGTGCCAA
851 AGAAGAAGAA CAACTAA
```

## Figure 47

1 MAQHPLVQRL LDVKFDTKRF VAIATHGPKN FPDAEGRKFF ADHFDVTIQA  
51 SILYVVVFG TKWFMRNRQP FQLTIPLNIW NFILAAFSIA GAVKMTPEFF  
101 GTIANKGIVA SYCKVFDFTK GENGYVWVLF MASKLFELVD TIFLVLRKRP  
151 LMFLHWYHHI LTMIYAWYSH PLTPGFNRYG IYLNFWVHAF MYSYYFLRSM  
201 KIRVPGFIAQ AITSLQIVQF IISCAVLAHL GYLMHFTNAN CDFEPSVFKL  
251 AVFMDTTYLA LFNFFLQSY VLRGGKDKYK AVPKKKNN

Figure 48

Host (plasmid)	334(pYX242)	334(pRET-21)	334(pRET-22)
Added Substrates	50 $\mu$ M GLA +	50 $\mu$ M GLA +	50 $\mu$ M GLA +
	50 $\mu$ M AA	50 $\mu$ M AA	50 $\mu$ M AA
Fatty Acid	%total fatty acid	%total fatty acid	%total fatty acid
C16:0	9.22	12.46	9.9
C16:1	0.09	0.18	0.13
C18:0	1.46	2.41	1.49
C18:1n-9	4.03	4.92	3.91
C18:3n-6	10.02	11.89	8.69
C20:3n-6	(1.28%)* 0.13	(11.1%)* 1.48	(19.4%)* 2.09
C20:4n-6	46.98	28.87	35.25
C22:4n-6	0	0	0
Total lipid (mg)	212	174	187
*% conversion=product/(substrate+product)			

Figure 49

1	ATGAACATGT	CAGTGTTGAC	TTTACAAGAA	TATGAATTCTG	AAAAGCAGTT
51	CAACGAGAAT	GAAGCCATCC	AATGGATGCA	GGAAAACCTGG	AAGAAATCTT
101	TCCTGTTTTTC	TGCTCTGTAT	GCTGCCTTTA	TATTCGGTGG	TCGGCACCTA
151	ATGAATAAAC	GAGCAAAGTT	TGAACTGAGG	AAGCCATTAG	TGCTCTGGTC
201	TCTGACCCTT	GCAGTCTTCA	GTATATTCGG	TGCTCTTCGA	ACTGGTGCTT
251	ATATGGTGTA	CATTTTGATG	ACCAAAGGCC	TGAAGCAGTC	AGTTTGTGAC
301	CAGGGTTTTT	ACAATGGACC	TGTCAGCAAA	TTCTGGGCTT	ATGCATTTGT
351	GCTAAGCAAA	GCACCCGAAC	TAGGAGATAC	AATATTCATT	ATTCTGAGGA
401	AGCAGAAGCT	GATCTTCCTG	CACTGGTATC	ACCACATCAC	TGTGCTCCTG
451	TACTCTTGGT	ACTCCTACAA	AGACATGGTT	GCCGGGGGAG	GTTGGTTCAT
501	GACTATGAAC	TATGGCGTGC	ACGCCGTGAT	GTACTCTTAC	TATGCCTTGC
551	GGGCGGCAGG	TTTCCGAGTC	TCCCGGAAGT	TTGCCATGTT	CATCACCTTG
601	TCCCAGATCA	CTCAGATGCT	GATGGGCTGT	GTGGTTAACT	ACCTGGTCTT
651	CTGCTGGATG	CAGCATGACC	AGTGTCACCTC	TCACTTTCAG	AACATCTTCT
701	GGTCCTCACT	CATGTACCTC	AGCTACCTTG	TGCTCTTCTG	CCATTTCTTC
751	TTTGAGGCCT	ACATCGGCAA	AATGAGGAAA	ACAACGAAAG	CTGAATAG

## Figure 50

1 MNMSVLTQLQ YEFKQFNEN EAIQWMQENW KKSFLFSALY AAFIFGGRHL  
51 MNKRAKFELR KPLVLWSLTL AVFSIFGALR TGAYMVYILM TKGLKQSVCD  
101 QGFYNGPVSK FWAYAFVLSK APELGDTIFI ILRKQKLIFL HWYHHITVLL  
151 YSWYSYKDMV AGGGWFMTMN YGVHAVMYSY YALRAAGFRV SRKFAMFITL  
201 SQITQMLMGC VVNYLVFCWM QHDQCHSHFQ NIFWSSLMYL SYLVLFCHFF  
251 FEAYIGKMRK TTKAE\*

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